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THE HANS GOLDMANN

Proctor Medal Award

PROCEEDINGS

of the

Association for Research in Ophthalmology, Inc.

First Mid-Winter Meeting

Edgewater Park, Mississippi

February 19, 20 and 21, 1959

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PROFESSOR HANS GOLDMANN

COMMENTS ON ACCEPTANCE OF THE PROCTOR MEDAL

HANS GOLDMANN, M.D.
Bern, Switzerland

It is an extraordinary honor for me that you found me worthy of the Proctor Medal. I feel singularly privileged to be the first continental European who has been thus recognized. When I examine myself—and such a high honor is due cause for self-examination—then I must confess that my contribution to ophthalmology is quite modest. I have worked in a very restricted segment of our science. I improved some instruments and helped to clarify some concepts and to extend their use to the clinic, concepts which were developed by Leber and Seidel in thorough animal experiments. I did no more than that, for which Herodotus has coined the classical definition of the task of every scholar: ἀλλήλοις λαμπάδα διαδιδόναι—"to take and transmit the torch."

Therefore, it seems appropriate to include in the honor which I receive today all the men who formed me, and all those who made my work possible: my teachers, my co-workers, and the country which has become my home. With Prof. Tschermak I learned physiology, especially physiologic optics. There are some of among you who were acquainted with him. He was a really remarkable man, a spirited personality, full of new ideas, but often far, very far, from the realities against which the practitioner and the teacher have almost daily to struggle.

Then I was fortunate to have as my second teacher the founder of the Swiss Ophthalmological Society, Prof. Siegrist of Bern, a man with real dedication to clinical work and enthusiasm for the teaching of young medical students. He was a critical but human and simple person, who hated nothing more than pseudoscience and professional arrogance. Again, it was he who introduced some of you to ophthalmology. It was a rare opportunity to work under these two men for some years. But it would be unjust not to mention two other men who also were my teachers. Elschnig introduced me to ophthalmology and filled me with enthusiasm for this specialty. He was an unequaled artist of intraocular operations. On the other hand I served an apprenticeship with Prof. Blaskowicz, the great and extremely modest master of extraocular surgery. Today I remember these four men with a feeling of profound gratitude.

But to be able to work quietly in a time of one of the greatest upheavals which Europe has seen in its long and agitated history was only possible for me because I had found a new home in Bern, in Switzerland. Here I found just the conditions I needed and the finest precision workers, who, as you know, helped me so much and so often with all their skill.

I accept this Proctor Medal in deep gratitude, not for me, but as an honor for all the people who gave me the opportunity for useful work.

University of Bern.

Presented at the 28th annual meeting of the Association for Research in Ophthalmology, Inc., Atlantic City, New Jersey, June 10, 1959.

BIOGRAPHICAL NOTE: PROFESSOR HANS GOLDMANN

The external circumstances of Professor Goldmann's life are quickly told. Born in Komotau (in what was then the Austro-Hungarian Empire, now Czechoslovakia) on November 21, 1899, he had his primary and secondary education in the town of his birth. From there he went to Prague to study medicine at the Germany University and obtained his degree of doctor of medicine in 1923. From 1919 through 1924, he was assistant to Prof. Tschermak in the Physiologic Institute, after which he became assistant in the University Eye Clinic in Berne, Switzerland, which was under the direction of Prof. August Siegrist. In 1927 he was made Oberarzt at the clinic and in 1930 became Privatdozent. Upon the retirement of Prof. Siegrist, in 1935, Goldmann became his successor as professor of ophthalmology and director of the University Eye Clinic. He has remained in this position ever since. His sojourn in Berne was interrupted in 1929 when he spent some time at the clinic of Prof. Blaskovics in Budapest and at the clinics in Vienna and Freiburg, Germany. In 1951 he was invited to make a lecture tour in Sweden; in 1953 he lectured in South America and in 1956 in Israel. That same year he was also invited to participate in the Second Glaucoma Symposium, held at Princeton, New Jersey, sponsored by the Josiah Macy, Jr. Foundation, and to deliver lectures in various cities of the United States.

A number of distinctions have come his way. He has been honored by membership in the Belgian, Greek, Portuguese, Brazilian and Peruvian Ophthalmological Societies and is an honorary member of the Ophthalmological Society of São Paulo and of the Swedish Medical Association. In 1946 he was awarded the Vogt Medal of the Swiss Ophthalmological Society; in 1950 he was the Doyne lecturer at the Oxford Congress, and now he has been awarded the Proctor Medal.

The true story of Goldmann's life is reflected not in its external circumstances or in the honors which have come to him but in his work and his accomplishments.

From the beginning of his career his keen mind was oriented toward scientific study. The first decade or so of his activity was largely devoted to investigations of the physiology and pathology of the crystalline lens. Among these investigations his studies on the etiology of the glassblowers' cataract attracted particular attention. Vogt had been of the opinion that this form of cataract resulted from a direct, specific effect of infrared radiation prevalently absorbed in the lens. Goldmann maintained that it was caused by an overheating of the lens owing to heating of the aqueous and more particularly of the iris. This difference of opinion provoked one of the few controversies of truly homeric proportions in modern ophthalmology. Invectives flew back and forth, and though Goldmann's views prevailed in the end, his friends were seriously worried about his academic future in Switzerland. Unnecessarily so: the good sense and independence of the Bernese authorities brooked no interference. Goldmann put the last link into the chain of evidence in a paper published in 1950 with Koenig and Maeder, in which it was shown that accurate determinations of the absorption curve of the excised lens of rabbit, calf and man revealed no specific absorption in the infrared. Indeed, these lenses absorbed less of this radiation than did corresponding thicknesses of water.

It may appear that an inordinate amount of ingenuity and time had been spent in the clinics in Berne and Zürich on the question of the genesis of the glassblowers' cataract, but these studies were, in fact, only a fraction of Goldmann's work on the lens. Stimulated by the interest of Siegrist in senile and experimental cataracts and by the availability of a new tool—the slitlamp—he contributed studies which greatly enlarged our

knowledge and understanding of the biology of the crystalline lens.

He demonstrated a new form of reversible osmotic cataract due to increased concentration of the aqueous, occurring in young rats in the pupillary area, following prolonged exposure of the eye to air. Studies on naphthalene cataracts, on X-radiation cataracts and on cataracts in parathyroidectomized animals gave many significant results. Goldmann found, for example, that subcapsular opacities in parathyroidectomized dogs and rats occurred only when there were also tetanic muscular symptoms and that the suppression of these symptoms prevented the formation of lenticular opacities. Some conclusions of general significance stand out. Lenticular opacities do not necessarily occur at the site of immediate action of the noxious agent. So-called "complicated" cataracts do not necessarily begin in the form of subcapsular opacifications. And, most important, the formation of the so-called zones of discontinuity in the lenses, that is, of optical surfaces separating two media of different indices of refraction, is due to temporary arrest in the appositional growth of the lens. The surface of the adult nucleus of the human lens increases in thickness with age and consists of three distinct layers of reflex zones, adolescent, adult and senile. The zones of discontinuity are thus a means of determining the temporal sequence of events in normal and abnormal lenses.

Although all of Goldmann's work is essentially biophysical rather than biochemical in nature, during the period of his preoccupation with the crystalline lens he also did significant work on ascorbic acid in its relation to lenticular metabolism and the blood aqueous barrier. He was assisted in this work by the late Wilhelm Buschke.

As a natural consequence of his studies of the lens which can be so beautifully investigated in the living organism, Goldmann became interested in improving the slitlamp. In co-operation with the firm of Haag-Streit he developed a model which was vastly superior to anything available at that time and which remained by far the best slitlamp until after the Second World War, when other models began to appear on the market, most of which incorporated in one form or another many of the basic features of the original Goldmann-Haag-Streit slitlamp. An ingenious photographic device, designed to photograph the anterior segment together with the slitbeam, and allowing, for instance, the measurement of the depth of the anterior chamber, has unfortunately never become commmercially available.

His slitlamp is not the only piece of instrumentation for which our profession is indebted to Goldmann. He has always been keenly interested in the development of new instruments and the improvement of old ones. As he himself stated in a remark at the Second Macy Conference on Glaucoma, his love for instruments stemmed from the days when he had to put together, in Tschermak's institute, an apparatus for color matching built 30 years previously by Hering, and of which only pieces were in existence.

Among the instruments which Goldmann has given us is the mirror contact lens which in conjunction with the prism reducing the angle between microscope and illuminating system allowed one for the first time to perform successful slitlamp gonioscopy. All further developments in this field stem from Goldmann's original lens. It has meant a great refinement in observation technique and even those who to this day do not recognize the value of the narrow slit technique and still maintain the superiority of the Koeppe type lens have greatly benefited by the advances which slitlamp gonioscopy has brought to us.

Goldmann did not stop at the chamber angle. He, as well as others, pushed biomicroscopy into the difficult area of the vitreous and to the eyeground. This work culminated in the magnificent volume which Goldmann, together with the late Dr. S. Schiff-Wertheimer, and that other master of biomicroscopy, A. Busacca, presented in

1957 as a report to the French Ophthalmological Society. This volume is one of the great achievements of the last two decades of ophthalmology. It is urgent that it should be made available in translation to all those who do not read French.

Neither did Goldmann stop at methods for the objective examination of the eye. His apparatus for the clinical examination of dark adaptation is rapidly becoming standard in many clinics; his apparatus for the objective determination of visual acuity, utilizing the phenomenon of optokinetic nystagmus, is still too little known. But the instrument which has brought probably the greatest theoretical and practical advances in the field of subjective examination of patients is his perimeter. It has given a sound, new basis for quantitative perimetry.

The latest contribution of Goldmann's to the methods of clinical examination is his applanation tonometer. This beautifully constructed instrument excludes, or reduces to a minimal degree, the error introduced into tonometry by the factor of the rigidity of the ocular coats. It thus permits one to come as close to a measurement of the true intraocular pressure as it is possible to attain in humans. The theory of the applanation tonometer has been developed by Goldmann in collaboration with Th. Schmidt, with whom he has also worked out a more exact determination of the rigidity factor. We have not even begun to reap the clinical fruits which are afforded us by the new devise.

The applanation tonometer is the capping stone of one line of Goldmann's work on the glaucoma problem which has occupied him during the last two decades, beginning in 1938 with his papers, conjointly with Bangerter, on gonioscopy in glaucoma.

The discovery of the aqueous veins by Ascher, and the evidence that these veins contained indeed aqueous humor, made the conclusion inevitable that aqueous is continually circulating from the eye through Schlemm's canal. This, together with the fact that fluid flows from the eye in a laminar not a turbulent way, allowed Goldmann to develop the mathematical theory of the maintenance of intraocular pressure, with the resulting establishment of the fundamental law of the intraocular pressure. This law states, according to Goldmann, that the outflow pressure of the aqueous is equal to its rate of flow per minute divided by the resistance to the outflow. Thus if it were possible to determine in every case the outflow pressure, that is, the gradient between intraocular pressure and pressure in the aqueous veins, and the rate of flow per minute, it should be possible to determine in each case the mechanism of increase in intraocular pressure.

Goldmann has greatly contributed to making this possibility a reality by developing methods for the determination of the episcleral venous pressure, the fluorometric measurement of the rate of flow of the aqueous and the measurement of the volume of the anterior chamber. His conclusion that the seat of the increased resistance in open-angle glaucoma is in the region between the anterior chamber and Schlemm's canal, though it has not gone unchallenged, has considerable practical importance and has been supported by much further research.

In his most recent work in the field of glaucoma Goldmann has turned to other aspects of the problem. He has shown with Gafner that increase in intraocular pressure causes a decrease in sensitivity in the arcuate area of the visual field, that is, the area where, in glaucomatous eyes, a Bjerrum scotoma is formed. Since he could also show that glaucomatous cupping does not simply occur from the increased pressure itself, he has proposed the hypothesis that the damage to the optic nerve in glaucoma arises from the fact that the vessels of the disc are particularly sensitive to an increase in intraocular pressure, because they are shunted by extraocular vessels.

Even the briefest survey of Goldmann's contributions to ophthalmology must not

overlook his work on various aspects of the physiology of vision. In his youth, he worked in Tschermak's laboratory on problems of color vision, and the physiology of vision has remained his first love. He studied with Schubert the effect of oxygen deprivation on the visual field, gave a most elegant proof of the retinal origin of the Stiles-Crawford effect and measured with R. Hagen the total refractive power of the living human eye by means of Rushton's method of X-ray visualization. His investigations dealing with the artificial production of gun barrel fields in normals, on the spatial summation in the light adapted retina, and his elucidation of the angioscotoma, all are major contributions not only to physiology but also to exact perimetry. Last, but not least, mention must be made of his studies on accommodation which demonstrated that the law of equal innervation applies to the intrinsic as well as the extrinsic ocular muscles.

This long list of achievements does not exhaust Goldmann's activities. A glance at his publications will show that he has not neglected the purely clinical aspects of ophthalmology to which he has frequently brought new and original thoughts. Thus he was one of the first to connect retrolental fibroplasia with excessive oxygen administration to premature babies. The most significant advance in the clinical field is likely to come from Goldmann's suggestion, elaborated by Witmer, that there is local antibody formation in the eye and that this local antibody formation may be used for the diagnosis of the etiology of uveitis. Very promising results have been obtained on the European content with this method of investigation and it is to be hoped that it may help us in this field, which is one of the most frustrating ones to every ophthalmologist.

The scope and value of Goldmann's contributions to scientific ophthalmology is truly remarkable. There are in medicine, as well as in all the sciences, men who have brilliant ideas. Then there are those men who bring new methods, and thus push backward the frontiers of knowledge. And lastly, there are those men who have brilliant ideas and the imagination and skill to devise new methods and put the ideas to the test. These are the great men of science and medicine. Goldmann is one of them.

There is little room in a life such as Goldmann's for hobbies. What hobbies he has are on an intellectual plane. He is an accomplished and even creative mathematician. He is a naturalist in the old sense of the word. Stars, animals, plants, all interest and fascinate him, and his knowledge about them is encyclopedic. In the delightful grounds of his vacation retreat, overlooking the Lago Maggiore in all its splendor, he grows a most unusual group of conifers which he has lovingly collected from all over the globe. But his curiosity extends to man, his past and his present, and to human activity in all its manifestations. This curiosity is, no doubt, the source of his vast erudition and of his sensitive appreciation of the visual art forms.

Goldmann is not gregarious. He is not a joiner, nor a "politician." He values his time too greatly. He is extremely exacting of his collaborators and even more so of himself. He can be very outspoken in a discussion. He readily spots nonsense and muddy thinking. But his friends know the essential warmth of his nature. The dreadfulness of some of the diseases we have to deal with affects him deeply. All his scientific work is in the last analysis directed to the one goal of helping to eradicate them. And so he is not only a scientist of the first order in our specialty but an outstanding surgeon and above all a great physician. Goldmann the physician motivates Goldmann the scientist. It is necessarily to both that the Proctor Medal is being awarded.

Hermann M. Burian.

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SOME BASIC PROBLEMS OF SIMPLE GLAUCOMA

THE PROCTOR MEDAL LECTURE

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The great diseases present many fascinating problems. Simple glaucoma is, in my opinion, one of these diseases. It is frequent in old people and because the number of old people in the population is increasing relatively and absolutely, it is a disease of increasing importance.

We are accustomed to treat diseases as pathophysiologic problems, that is, basically, as scientific problems. Because science does not exist free of supposition, we frequently make working hypotheses, examine them in the light of experiment and observation and see within what limits they obtain. But when I now, from these generalizations go on to our concrete disease, simple glaucoma, you soon will understand that we have not the right to employ only measures of science.

Two opinions oppose each other concerning simple glaucoma. One point of view is that simple glaucoma is a disease in which an increased intraocular pressure damages the optic nerve, slowly bringing about blindness. The other opinion holds that simple glaucoma is a disease of the whole person, especially the central nervous system; a disease which produces neurovascular disturbances in the optic nerve, slowly bringing about blindness. Added to the neurovascular disturbances in the optic nerve are more or less grave disturbances of the regulation of intraocular pressure which manifest themselves primarily in strong fluctuations of the intraocular pressure. However, even without any increase of the intraocular pressure, the disease eventually results in blindness. It may be that an increase of the intraocular pressure is unfavorable for the course of the disease.

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One sees that these two opinions are very different from each other. Such divergent opinions are often encountered in scientific problems which are not definitely solved. Fifty years ago, for instance, there was a great controversy as to whether an elementary electric charge existed or not. Is the controversy concerning simple glaucoma and that concerning the elementary charge of the same kind? I think the answer is no.

In the case of the controversy regarding the existence of the electron, the scientific workers were only obliged to pursue their search. It was not urgent. It did not matter if the problem was solved today, or in 10 years, or in 20 years. When it was solved, new questions arose which led to new knowledge.

In our problem of disease, there is superimposed something specifically medical that distinguishes it from a simple scientific problem and makes controversies especially bitter. The disease is there and we have to do something about it. We have to do what is the best under the circumstances of our restricted knowledge. You see I purposely have not yet professed to follow one or the other opinion. I have not yet applied scientific methods.

In spite of this, the question of the proper handling of the case is posed. One of the opinions concerning simple glaucoma demands that we normalize the tension during 24 hours of the day. The other does not demand this. The second opinion lightens the burden of the doctor so far as regulation of the tension is concerned; he need only influence the intraocular pressure a little bit by drugs, and later on maybe operate. When the state becomes worse, the doctor is never responsible; it is the nature of the disease and its known course.

How must the conscientious doctor manage this disease even if both opinions of which I speak are equally plausible, but the decision between the two has not yet been reached? I think that it is absolutely clear how he must behave. He must act as if the first opinion were correct, that is, in simple glaucoma he must keep the tension normal around the clock. Only in this way is it guaranteed that the doctor adheres to the chief basic law of the medical art—primum non nocere—firstly, do no damage.

I would not have insisted on bringing up this question had I not seen many people who became blind because of a prolonged half-hearted treatment fostered by this second opinion. It is, as you see, a moral question, the question of how we medical men have to act in order to keep our conscience pure, which must be answered before we venture on the problems of pathophysiology of simple glaucoma.

We have considered the ethical problem and given an unequivocal answer. Now a philosphical problem imposes itself. When the nervous system is implicated in the explanation of a pathologic entity it is very easy, by means of general reflections and introduction of analogies, to give apparently satisfactory explanations. Therefore I wish to insist: neurovascular mechanisms can so glibly explain every phenomenon that they should be only used for explanation if either a detailed nervous mechanism has been found for a special phenomenon, or if no possibility for explanation remains other than the supposition of such nervous mechanism.

Expressing this in another way, I say that the man who advocates the neurovascular causation of simple glaucoma has the obligation to prove this allegation. The deus ex machina of the vegetative nervous system should only be allowed to appear when every other explanation has proved futile, and not a priori.

Now we are at the point where we can begin to speak of basic problems of the pathophysiology of simple glaucoma.

We know with certainty that the aqueous has bulk outflow from the anterior chamber through Schlemm's canal and the aqueous veins. There is a simple expression which describes the interrelationship between this flow and intraocular pressure[1]: $\Delta P = R.F.$ In this formula ΔP is the pressure head between a determined level and the intraocular pressure. F is the rate of flow of the aqueous, and R is a proportionality factor, the resistance. If, at a certain time and in a particular eye, F is known, then R depends on the zero point chosen for the outflow system: If ΔP is taken equal to P_o minus P_v, where P_v is the episcleral venous pressure, then R represents the resistance between the anterior chamber and the point in the episcleral venous system with the pressure P_v.

If there are the same rate of flow of aqueous and the same episcleral venous pressure but a higher intraocular pressure, then an increased outflow resistance is implied. From the formula it also follows that intraocular pressure depends upon episcleral venous pressure. This relationship has been repeatedly demonstrated.[2] It does not follow from the formula that in a given eye F is necessarily a constant, which cannot change when, for instance, the resistance changes. Nor does it follow from the formula that an increase of intraocular pressure—say by compression of the globe—does not change the rate of flow. Nor does it follow that the several factors are constant during the period of measurement. Moreover, if we determine by experiment F (the rate of flow and calculate the resistance then ΔP (the difference between intraocular pressure and the selected zero point of the outflow system) determines the value of this resistance. That is, if a method is used which directly determines resistance, then the associated zero point of the system must be found before the rate of flow can be calculated from our formula.[3]

I have explained to you in brief all of the principal difficulties implied in the determination of the parameters of the basic formula for the outflow. Two methods for the study of the pressure-flow-resistance system of the eye have been utilized. In one[4] you determine the rate of flow and the pressure head in the undisturbed eye and calculate the resistance; in the other[5] you determine the resistance by compression of the eye, measure the pressure head and calculate the flow. The first method takes a long time —more than an hour—for the determination of the rate of flow. It presupposes, as you see, a constancy of all of the values during this time. The second method, tonography, presupposes that the compression of the eye changes neither the rate of flow, nor the resistance, nor the zero point of the pressure head, nor the value of the equilibrium pressure, P_o. It is short and therefore more interesting for clinical use than the first method. However, if we are to use tonography, we must attempt to diminish or remove as many of the sources of error as possible.

Tonography, according to the procedure of Grant,[5] utilizes the Schiøtz tonometer, the P_t and V values of Friedenwald and Friedenwald's law of scleral rigidity. Friedenwald has the immortal merit of having clarified the theoretical basis of tonometry,[6] and largely through his effort we have today standardized Schiøtz tonometers and a nomogram which yields P_o values which are in reasonable agreement with what we believe to be fact. However, I think you have all been disturbed in recent times because different authors[7] have presented reports which demonstrate that this law of scleral rigidity and even the P_t and V values may not be exact.

While these findings do not lessen the usefulness of the Schiøtz tonometer for measurement of intraocular pressure, they raise questions regarding the exact interpretation of tonography which depends upon these very values. For instance, we developed a method to examine whether, during the tonographic increase of pressure, secretion remained constant.[8] We found that it decreased under the supposition that the P_t and V values and Friedenwald's law which we employed were correct. There was always a doubt about these basic features of the method. Only a radical change of the method can free us from this dependency upon questioned tables and formulas. We need a method in which the errors can be better estimated. Therefore, we are trying to develop a tonography based on applanation.[9]

By employing different areas of applanation it is posible to ascertain a segment of the P-V curve of the living eye. In the region between 10 and 35 mm. Hg the curve seems to be very well represented by Friedenwald's rigidity law. The values obtained are in good accordance with the known values if one takes the volume displaced by the tonometer as the total volume displaced. And there a new difficulty comes in, because the volume displaced by the tonometer is not the total volume displaced. Even this phrase is not correct. In tonographic experiments you try to compare *deformation* of the globe by the tonometer characterized by a volume with real *elimination* of liquid out of the eye. The only common denominator is that you express both in volume, but deformation and elimination are essentially different things.

For the Schiøtz tonometer Prijot[10] found the real amount of displaced liquid in the living eye to be 1.5-1.7 times the volume of indentation. For applanation the volume is different. It was necessary to develop the theoretical basis for a better determination of the liquid eliminated. We found a suction method promising.

Now, after having defeated all these difficulties we can begin with applanation tonography. If one raises the intraocular pressure to a definite level by pressing on the equator of the eye and checks the pressure by ap-

planation measurements on the cornea, for a definite time, at the end of compression one can determine the volume lost by means of applanation methods and the previously determined P-V curve. Now the outflow resistance can be calculated if we know P_o.

What is P_o? You all know that repeated tonometry with the Schiøtz tonometer, though very brief, lowers the intraocular pressure. One speaks of massage effect. Various authors, especially Stocker,[11] have shown that the lowering of pressure may persist, and may also be detected in the other eye. Even more impressive is what is seen in applanation tonometry. Here the tension is increased during tonometry by less than one mm. Hg. In spite of this minimal increase, the intraocular pressure of many normal persons diminishes during repeated tonometry some two or more mm. Hg in three or four minutes. In some subjects the decrease continues for longer than this.

It can be shown that the decrease in pressure is real and not merely a change in corneal consistency. Moreover, if measurement is continued, the decreased tension level is maintained, but may readily be disturbed for short periods by emotional excitement.

What does this mean for tonography? There are three possibilities:

1. It is a vascular effect, a decrease of volume of the globe by vasoconstriction. By such an effect P_o, the intraocular pressure of equilibrium is not changed, and in tonography one should employ the first measured P_o value.

2. It is a vascular or muscular effect, but one where the change is not a decrease of pressure but is an increase of tension, perhaps due to the manipulations and preparations before tonometry, and that the decrease is a reestablishment of equilibrium. In this case P_o is not the first, but the last stable value of the tension.

3. The decline in pressure is the result of change in one or more of the basic factors of outflow—a decrease in secretion, a diminution of episcleral venous pressure, and so on. In this case also the final value of P_o must be used in the tonographic calculations.

What possibility is there of distinguishing between Case 1 on one hand and Cases 2 and 3 on the other hand? If one considers the rate of change of volume of the eye with change in pressure $= \frac{dv}{dp}$, one sees that this corresponds, in the electrical analogy, to a capacitance. On the discharge of a capacitance through a resistance, capacitance times resistance has the dimensions of time. Thus, a capacitance—resistance system may be characterized by a time constant, the time required for the system to dissipate 63 percent of its charge.

The human eye constitutes such a capacitance—resistance system and the time constant of the normal eye may be calculated to be approximately four to six minutes. But the pressure remains stable at the lower level for over 15 minutes and this suggests that the lower pressure value is the real P_o of equilibrium. What here is demonstrated as characteristic with the applanation tonometer must be true similarly with Schiøtz tonography. Much of the rapid decreases of pressure seen in the first part of many tonographic curves which we called "creep" is not creep but depends on the preparation of the patient. It confirms to a great extent what Grant[12] has insisted upon. But if one does not wait until P_o stabilizes before performing tonography—and this stabilization may take several minutes and cannot directly be deduced from the tonographic curve—quite erroneous P_o values will be taken for the calculation of facility, even if the first part of the tonographic curve is not used for calculation.

As to the results of our work with applanation tonography, it is very early in the game to say much. So far, even in very early cases of simple glaucoma, we have found increased resistance. Perhaps in very early glaucoma, nervous disturbances of homeo-

stasis may play a role. We have no support for this, but our material is small, so we must leave the question open. We can however confirm with this method that in simple glaucoma of longer standing the cause of the increased tension is an increase of the resistance of the outflow channels of the angle. The instability of the pressure of many of these cases has at least three purely mechanical reasons:

1. The P-V curves show that the capacitance of the normal eye is much greater than the capacitance of a glaucomatous eye on account of the lower pressure level. So, equal volume changes produce a greater pressure change in glaucomatous than in normal eyes.

2. The time constant of a glaucomatous eye is higer than that of a normal eye, because resistance increases much quicker than capacitance decreases; therefore every disturbance of equilibrium needs more time to be compensated.

3. Every change in flow must cause greater pressure changes in a eye with high resistance than in one with a low one.[13] The interference of a nervous mechanism[14] is therefore not necessary for the elucidation of the wide diurnal swings of tension seen in simple glaucoma.

Up to this time we have spoken about the various aspects of the problems of simple glaucoma, but a most important facet is still missing. People suffering from uncontrolled simple glaucoma long enough become blind. The visual field decays as a rule in a very characteristic fashion and a specific atrophy of the optic nerve, the glaucomatous excavation, develops. Certain investigators, and not unimportant ones, hold the opinion that this glaucomatous atrophy is a manifestation of a general disease and to a great extent is independent of the increase of intraocular pressure.

How is it possible that such an opinion could find serious defenders?

There is no doubt that in secondary glaucoma and in angle-block glaucoma the high pressure is the cause of the glaucomatous excavation, the atrophy of the optic nerve and the deterioration of the visual field. The chief arguments for the independence of the disease of the optic nerve from the ocular hypertension are the following:

1. In secondary glaucoma high intraocular pressure may be withstood for months without visual field defect. On the other hand, in simple glaucoma a small increase of tension is correlated with very severe losses in the visual field.

2. Cases of simple glaucoma with grave damage to the visual field continue to decay to virtual blindness even after normalization of the tension.

3. It is said that cases of modest but definitely pathologic ocular hypertension exist where the eye never shows a visual field defect, and on the other hand there are cases with normal tension but with glaucomatous atrophy and field loss. To this third point we say: cases which we have seen with apparently modest hypertension for decades and without visual field damage, were eyes with a high coefficient of scleral rigidity and normal pressure. The greater part of so-called glaucomas without hypertension were eyes with extremely low scleral rigidity. There are, in fact, cases with normal tension, very slowly progressing field defects and glaucomatous excavation of the optic nerve, but these are very rare. These rare cases need further study.

The other observations which are at the base of the doubt about the correlation of loss of visual function and intraocular pressure can be treated together. Secondary glaucoma and angle-block glaucoma are states in which we can often exactly indicate the day when the increase in pressure began. In simple glaucoma this has not been possible. Now, thanks to the extremely interesting work of Leydhecker,[15] we can say, in the main how much time elapses between the beginning of a simple glaucoma as an increase of pressure and the beginning of the visual field defect. Leydhecker has ex-

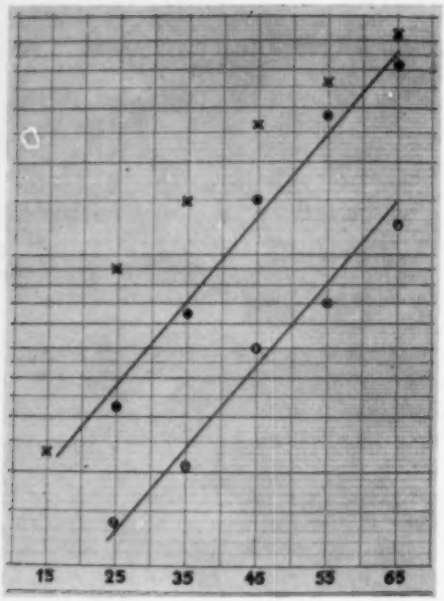

Fig. 1 (Goldmann). For explanation see text.

amined 19,000 eyes of so-called normal people with a standardized Schiøtz tonometer.

He found 427 people with a pressure higher than 20 mm. Hg. These 427 people were treated as glaucoma suspects and were subjected to a battery of tests, including diurnal pressure curves, provocative tests, ophthalmoscopy, perimetry, and a modified tonography which Leydhecker[16] previously had found highly successful in distinguishing between normal persons and cases of early glaucoma. Persons in whom at least two of the additional tests confirmed the suspicion of glaucoma were classified as glaucoma patients.

From these proven cases Leydhecker further selected those with well-established field defects.

If one puts the number of the diseased as logarithm on the ordinate and their age on the abscissa, very interesting plots can be obtained (fig. 1).[17] The suspected cases (crosses, fig. 1) represent an irregular group, as is to be expected. The confirmed simple glaucomas have (full circles, fig. 1), on a semilogarithmic representation, a good linear correlation. This means that their number increases with age according to a curve of organic growth.

Glaucomas with visual field defect (open circles, fig. 1) also lie on a straight line. The two lines are parallel, but displaced from one another by approximately 18 years. What does this mean? It means that increased intraocular pressure precedes visual field defects. Both have the same rate of increase in incidence. Therefore, in all probability, the decay of the visual field is the result of the increase in pressure but, from the beginning of the pathologic increase of pressure until the manifestation of the visual field loss, there is a lapse of more than one decade. The simple glaucoma with severe field loss is therefore the end state of a disease which began decades earlier.

Is it then to be wondered at, that in secondary glaucoma where the day of onset is exactly known, an increased tension may be withstood without field damage for a few months? I think that the findings of Leydhecker invalidate the evidence of these cases for a statement that intraocular pressure has nothing to do with visual field decay.

What concerns us now is the sad fact that in glaucoma with very grave field loss, blindness often can not be avoided, even when the pressure is normalized. I will tell you a story pertinent to this:

A man was discharged from military service in 1940 because of vertigo and slight unilateral diminution of hearing. In 1954 he went to an ophthalmologist because he had headaches and, from time to time, somewhat blurred vision. Papilledema was found, with normal visual acuity and enlargement of the blindspots. A neurologist made the diagnosis of tumor of the cerebellopontine angle and advised operation, which was declined. In November, 1956, the condition of the patient was objectively virtually unchanged. But shortly afterward, his visual acuity seriously diminished. He saw 0.5 (20/40) and 0.3 (20/70) and his visual fields were very restricted. The choked discs were atrophic. Now the patient consented to the operation. The surgery went well, but the patient's vision went down and now he is totally blind.

Everyone of you knows such cases. No one doubts the connection between the loss of vision and the intracranial hypertension caused by the tumor. When the cause of the hypertension is removed early, the choked disc disappears without damage to the optic nerve. But when the atrophy has begun then, in many cases, even removing the cause can no longer interfere with the destruction of the optic nerve.

Now we see that what we have called the full picture of simple glaucoma is nothing else but a late and often final phase of evolution—a final, often a moribund state, the result of years of insult to the optic nerve. At this stage normalization of the tension may or may not rescue the few remaining neurons.

On the other hand it is clear that there is plenty of time during which one can diagnose simple glaucoma. Systematic measurement of tension in persons over 40 years of age every three or four years when they have the regular check for their presbyopia should suffice to detect almost all cases of simple glaucoma before great damage is done to the function of the eye, and permit early institution of treatment. Long experience has proved to me and to others that early and adequately continued strict normalization of tension avoids decay of the visual field in almost every case.

It would appear that we have now reached the point where the problem of simple glaucoma seems to be resolved in its principles:

Simple glaucoma is a disease in which the increased intraocular pressure caused by an increase of the resistance of the outflow system of the eye slowly damages the nerve and finally causes blindness. Is this really so?

I think that this definition is valid for the fully developed simple glaucoma. Is it true at the start of simple glaucoma? I do not know.

If somebody says that in the group of patients with beginning glaucoma simplex there is a disturbance of the regulation which leads to an increase of resistance which may be reversible at the start and becomes irreversible later on, I do not believe that our actual methods of examinatioin can refute such a hypothesis. The proof or the refutation of such a possibility would be of extreme practical importance, because it would raise the question of the reversibility of a commencing glaucoma.

On the other hand, is it not true that the decay of the visual field in the late state of simple glaucoma (even if the tension is normalized) proves that, at a certain point, the disease of the optic nerve becomes independent of the intraocular pressure?

Again new and important questions arise.

Is the transition from the state of the disease, which is dependent on the pressure, to the state where it becomes independent, sudden—or does the limiting pressure value which is just damaging, slowly diminish during the course of the disease until at last there is no pressure at all compatible with the survival of the rest of the optic nerve?

All these questions require a method that will determine in every single case the limiting pressure which the individual optic nerve can withstand continously without damage. Only when we have resolved these borderline problems in both early and late glaucoma can we evaluate to a certain extent the whole pathophysiology of simple glaucoma. Even then in the process of their solution there may arise basic problems at present beyond our conception.

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OPHTHALMODYNAMOMETRY: A DIAGNOSTIC AID IN CEREBROVASCULAR DISEASE*

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Ophthalmodynamometry is a recently recognized aid in the diagnosis of cerebrovascular disease. It should enhance the role of the ophthalmologist in neurologic diagnosis and provide indications for carotid vascular surgery. More than 40 years ago Bailliart[1] described his technique of ophthalmodynamometry, which made possible estimation of diastolic and systolic blood pressures in the central retinal artery. Occlusions of the carotid artery will usually cause lowering of the blood pressure in its first branch, the ophthalmic artery, and in the central retinal artery. Therefore, comparison of the blood pressure in the artery of each eye by ophthalmodynamometry is a convenient bedside method for detecting occlusion of the carotid artery.

The increasing importance of early diagnosis of occlusion of the carotid artery is due to a number of factors. First, surgical

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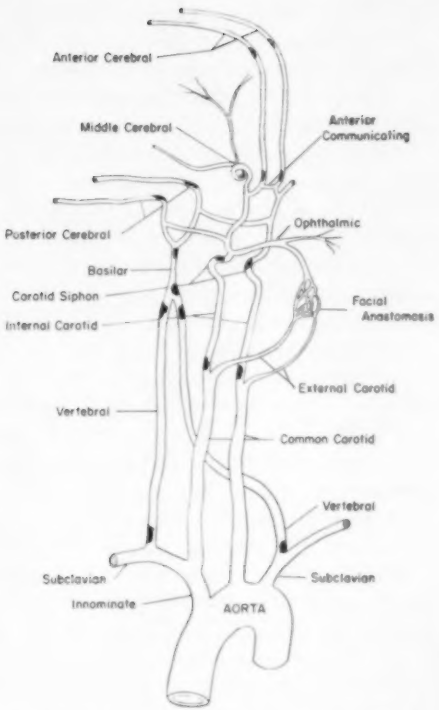

Fig. 1 (Schimek and Beallo). Diagrammatic representation of common sites of atherosclerotic involvement and occlusion of the arteries. (Courtesy of Jackson.[3])

cure of vascular occlusions has become feasible only recently. Secondly, as the life span of the population has been lengthened, the incidence of "strokes" due to cerebral thromboses and cerebral hemorrhages, as well as intermittent cerebrovascular insufficiency, has increased. The last condition is our concern here. Hutchinson and Yates[2] showed that it is caused by lesions of major vessels in the neck in 40 of 83 consecutive patients with atherosclerotic cerebrovascular insufficiency.

Figure 1 shows the commonest sites of atherosclerotic involvement of the arteries.[3] Occlusion at the proximal segment of the carotid artery is common and can be attacked surgically. Since the brain is only two percent of the body weight but receives one sixth of the cardiac output and is completely dependent on glucose and oxygen, consuming nearly one fifth of the oxygen used by the body, the importance of prompt surgical treatment for carotid obstruction is obvious. Occlusion of a carotid artery can cause contralateral hemiplegia, seizures, and ipsilateral blindness. However, if the collateral circulation is good or if the occlusion is partial, it may cause either no symptoms at all or else gradually developing or recurring symptoms.

In such cases, diagnostic information can be obtained by (1) palpating the arteries in the neck for absence of pulsation of the carotids, (2) digital pressure to the unaffected carotid artery to cause syncope or other symptoms by reducing the collateral circulation, (3) arteriography (figs. 2 and 3), and (4) ophthalmodynamometry. Digital pressure on the carotid arteries and arteriog-

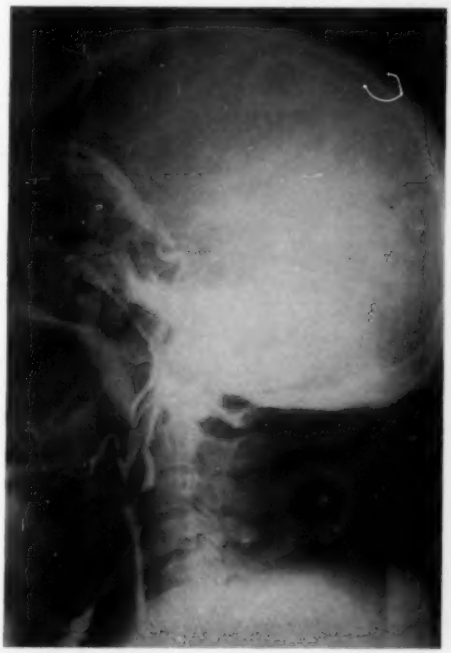

Fig. 2 (Schimek and Beallo). Carotid arteriogram showing partial obstruction of the internal carotid with filling of external and internal carotid systems. (Courtesy of Jackson.[3])

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OPHTHALMODYNAMOMETRY: A DIAGNOSTIC AID IN CEREBROVASCULAR DISEASE*

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Ophthalmodynamometry is a recently recognized aid in the diagnosis of cerebrovascular disease. It should enhance the role of the ophthalmologist in neurologic diagnosis and provide indications for carotid vascular surgery. More than 40 years ago Bailliart[1] described his technique of ophthalmodynamometry, which made possible estimation of diastolic and systolic blood pressures in the central retinal artery. Occlusions of the carotid artery will usually cause lowering of the blood pressure in its first branch, the ophthalmic artery, and in the central retinal artery. Therefore, comparison of the blood pressure in the artery of each eye by ophthalmodynamometry is a convenient bedside method for detecting occlusion of the carotid artery.

The increasing importance of early diagnosis of occlusion of the carotid artery is due to a number of factors. First, surgical

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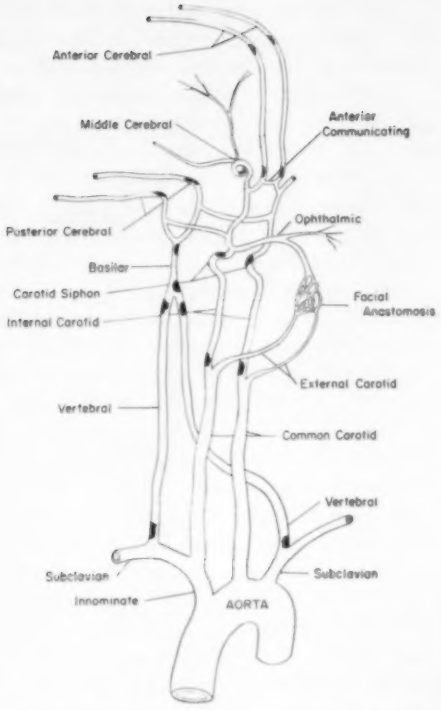

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Figure 1 shows the commonest sites of atherosclerotic involvement of the arteries.[3] Occlusion at the proximal segment of the carotid artery is common and can be attacked surgically. Since the brain is only two percent of the body weight but receives one sixth of the cardiac output and is completely dependent on glucose and oxygen, consuming nearly one fifth of the oxygen used by the body, the importance of prompt surgical treatment for carotid obstruction is obvious. Occlusion of a carotid artery can cause contralateral hemiplegia, seizures, and ipsilateral blindness. However, if the collateral circulation is good or if the occlusion is partial, it may cause either no symptoms at all or else gradually developing or recurring symptoms.

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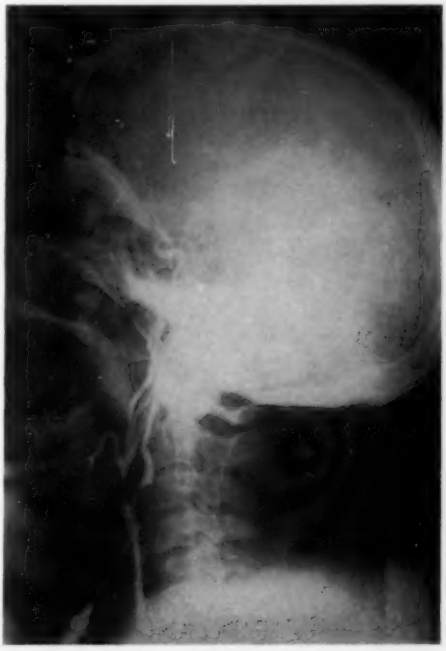

Fig. 2 (Schimek and Beallo). Carotid arteriogram showing partial obstruction of the internal carotid with filling of external and internal carotid systems. (Courtesy of Jackson.[2])

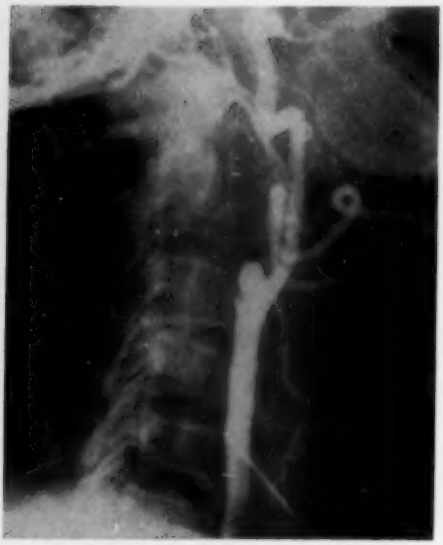

Fig. 3 (Schimek and Beallo). Carotid arteriogram showing obstruction at the origin of the internal carotid artery and filling of the external carotid system. (Courtesy of Jackson.[3])

raphy entail definite dangers, whereas ophthalmodynamometry is a safe procedure of considerable diagnostic value.

The technique of ophthalmodynamometry has been adequately described in the literature.[4,5] The ophthalmodynamometer is a spring-loaded plunger rod which is calibrated to measure pressures ranging from 10 to 150 gm. It is equipped with a footplate which is placed against the conjunctival surface of the eye at the insertion of the lateral rectus muscle. The ophthalmodynamometer is pressed against the eye with increasing pressure until the central retinal artery on the disk is noted to have a collapsing pulsation. At this point the intraocular pressure has been raised to exceed slightly the diastolic level of the blood pressure in the retinal artery; the finger is applied to the brake on the instrument and the reading is obtained. When the measurements are made in the patient's right eye, the ophthalmodynamometer should be held in the examiner's left hand and the ophthalmoscope in his right hand. For the left eye, the examiner should hold the ophthalmodynamometer in the right hand and the ophthalmoscope in the left.

To obtain the systolic pressure, the pressure with the instrument is increased until all visible arterial pulsation ceases. The diastolic pressure should be taken four or five times in each eye to insure accuracy. The systolic pressure should be obtained only after all the diastolic readings are made because a more pronounced lowering of ocular pressure occurs after determination of the systolic readings. The pupils should be dilated for maximum ease of performance and the eyes may be anesthetized topically.

The direct readings are in grams; this affords comparison between the retinal blood pressures of the two eyes. Results were recorded in this manner throughout the present study. The readings can be converted into millimeters of mercury by a conversion table[6] which also must take into account the intraocular pressure. The intraocular pressure should be determined in all cases for maximum accuracy. It is not necessary to convert the ophthalmodynamometer readings to millimeters of mercury unless the intraocular pressure readings are grossly unequal.

Results of other studies

Since the central retinal artery is a direct branch of the ophthalmic artery, which is the first branch of the internal carotid artery, occlusion of one internal carotid artery would be expected to result in a much lower pressure of the central retinal artery on the occluded side as compared with the patent side. The literature indicates that this is undoubtedly true in most cases of obstruction of the carotid artery. For example, in Wood and Toole's series[7] of five patients with complete occlusion of the carotid artery, four had more than 36-percent reduction in the diastolic pressure of the central retinal artery on the obstructed side, and one patient had a diminished diastolic pressure of 25 percent in the central retinal artery. Van Allen and as-

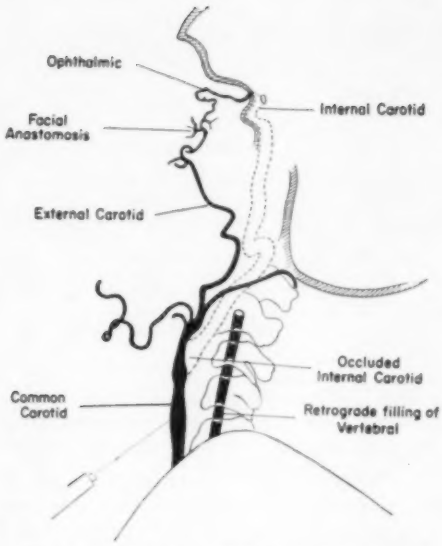

Fig. 4a (Schimek and Beallo). Diagrammatic representation of obstruction at the origin of the internal carotid artery with the external carotid system open and anastomoses functioning between these two systems. Blood from the external carotid by way of the facial artery and the facial anastomoses with the ophthalmic artery anastomose with and backfill the internal carotid system. (Courtesy of Jackson.[3])

sociates[8] described three cases of complete occlusion of the carotid artery, with the pressure in the central retinal artery lowered from 30 to 49 percent on the occluded side. However, in other studies less noticeable differences in the diastolic pressures of the central retinal artery are reported despite occlusion of the carotid artery on one side. For example, Hyman, Karp and Bloor[9] reported the following percentage decreases in the diastolic pressures of central retinal arteries ipsilateral to complete carotid occlusions. Among seven patients with carotid occlusions, three had from 36 to 59 percent decrease in the central retinal artery ipsilateral to the occluded carotid, three had only a 17 to 22 percent decrease, and one patient had merely a four-percent decrease. In a group having ligation of the carotid artery, one had only a five-percent decrease, whereas four others had more significant decreases from 19 to 42 percent in the pressure of the central retinal artery ipsilateral to the partial occlusion.

Figure 4-a shows diagrammatically the anastomosis between the external carotid and internal carotid systems by way of the facial artery and the facial anastomoses with the ophthalmic artery and the internal carotid system. Figure 4-b shows a carotid arteriogram in which the internal carotid artery was occluded with dye passing from the external carotid into the facial artery and through anastomoses into the ophthalmic artery to backfill the internal carotid system. Obviously, in such cases with excellent collateral circulation the decrease of arterial pressure will not be as great as one might expect. On the other hand, some normal persons will have considerable variation in pressure of the central retinal artery.

Again, in 13 patients with no evidence of carotid occlusion, Hyman and associates[9] reported a difference of three percent or less between the diastolic pressures of the cen-

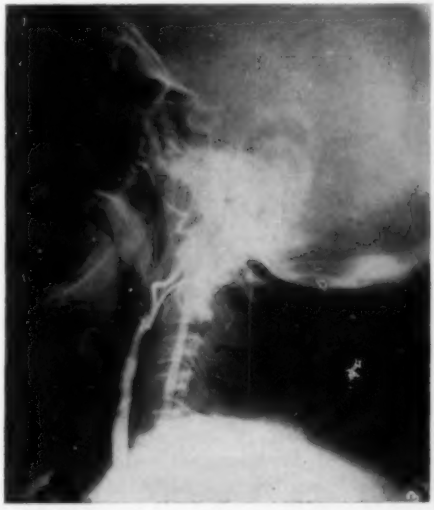

Fig. 4b (Schimek and Beallo). Carotid arteriogram showing actual internal carotid occlusion, with dye passing from the external carotid into the facial artery and through anastomoses (facial) into the ophthalmic artery to backfill the internal carotid system. (Courtesy of Jackson.[3])

TABLE 1

COMPARATIVE RETINAL ARTERY DIASTOLIC PRESSURES WITHOUT CAROTID OCCLUSION

Case	Diastolic Readings (in gm.)	Difference (percent)	Open by Angiography	Final Diagnosis
1	60-60	0	Yes	Basilar insufficiency
2	33-33	0	—	—
3	75-75	0	Yes	Subarachnoid hemorrhage
4	80-80	0	Yes	Cerebral atrophy
5	25-25	0	—	Basilar insufficiency
6	52-52	0	—	? Hysteria
7	90-90	0	—	Cerebral atrophy
8	62-59	4.8	—	Basilar insufficiency
9	70-65	7	—	Basilar insufficiency
10	60-50	16	Yes	—
11	60-50	16	Yes	Cerebrovascular accident

tral retinal artery in seven, and a difference of four percent in four. Two patients, however, had a 16-percent and an 18-percent difference respectively. Therefore, it is likely that the difference between the pressures of the central retinal artery must usually be of the magnitude of 25 percent or more in order to be significant. This will probably allow a small overlap between some patients with normal carotid arteries and some with obstructions of the internal carotid artery but with excellent collateral circulation.

Some investigators[8, 9] have noted that ophthalmodynamometer readings of the systolic pressures are of great value in partial occlusion of the carotid artery, and in equivocal diastolic pressure readings. We have not routinely obtained systolic readings because of the possible danger of inducing permanent closure of the central retinal artery. This complication would seem possible in a patient with arterial vascular disease, just as digital occlusion of the carotid artery may be dangerous. However, such a complication must be rare indeed. We have been advised that one such occlusion of a central retinal artery with permanent visual impairment was precipitated in a medical center where many ophthalmodynamometer determinations of systolic pressures are routine. In another medical center, transient occlusion of the retinal artery occurred but subsided without permanent damage. Our policy has probably been ultraconservative in determining only the diastolic pressures routinely. Systolic pressures were determined only in exceptional cases, and then with extreme care to exert pressure for as brief a period as possible.

RESULTS OF PRESENT STUDY

The following studies were performed on patients at the Ochsner Foundation Hospital during the past year. Ophthalmodynamometry was usually performed on patients in whom carotid occlusion was suspected. The cases in Table 1 were finally diagnosed clinically as having no carotid obstruction. Some of these patients were demonstrated by angiography or by exploration to have no carotid obstruction. The majority had essentially no difference between the diastolic pressures of the central retinal arteries. The following are interesting examples of some patients in whom the carotid vessels were diagnosed as normal:

CASE 3

This 42-year-old man was admitted to the Neurosurgical Service of Ochsner Foundation Hospital with a severe sudden left frontal headache. The clinical impression on admission was intracranial aneurysm with rupture. The ophthalmodynamometry readings were equal in both eyes, 75/75 gm. Arteriograms revealed no evidence of intracranial aneurysm and no evidence of occlusion of the carotid arteries. The patient was discharged 10 days later with the diagnosis of subarachnoid hemorrhage.

TABLE 2

COMPARISON OF RETINAL ARTERY DIASTOLIC PRESSURES IN COMPLETE UNILATERAL CAROTID OCCLUSION

Case	Diastolic Readings (in gm.)		Decrease (percent)	Occlusion Shown by	
	Open	Occluded		Angiography	Operation
12	36	24	33	—	Tied
13	80	53	34	Yes	Yes
14	70	60	14	Yes	Yes
15	46	39	15	Yes	—
16	65	42	35	Yes	Yes
17	74	55	25	—	—
18	No consistent readings		—	Yes	Yes
19	85	60	29	Yes	Yes
20	45	28	37	Yes	Yes

CASE 7

This man was admitted to the Neurosurgical Service with a history of sudden weakness and numbness in the right hand once five years before admission and again 10 days before, lasting several hours each time. Ophthalmodynamometer readings were 90 gm. bilaterally. Physical examination revealed a hesitancy of speech, a weakness in the right lower extremity, and some spasticity on the right. Occlusion of each carotid by digital pressure caused no change. A pneumoencephalogram demonstrated dilated lateral ventricles, a large amount of air over the hemispheres, and flattening of the gyri, compatible with a diagnosis of cerebral atrophy.

CASE 9

This man had ataxia, dizziness, dysarthria and transient cranial nerve involvement with diplopia. Ophthalmodynamometer readings were 70 gm. on the right and 65 gm. on the left, a difference of about seven percent. The clinical impression of disease of the basilar artery was substantiated by a vertebral arteriogram showing a plaque in the basilar artery. Anticoagulant therapy was prescribed and the patient was discharged.

CASE 10

This woman had a history of intermittent "blackout" spells with the left eye, the first spell occurring ten months before admission and lasting five minutes, and three similar episodes recurring during the previous six months. Ophthalmodynamometer readings were 60 gm. in the right eye and 50 gm. in the left eye, a difference of 16 percent. On carotid arteriography the carotid system on both sides was patent, although there was some irregularity of the left internal carotid. However, the left ophthalmic artery was never visualized. Since the ophthalmic artery did not fill during angiography, and because of the intermittent blind spells, this patient may have had an obstruction close to the orifice of the ophthalmic artery.

Table 2 shows comparative pressures in the retinal artery with complete carotid occlusion on one side. It will be noted that there is usually a significant decrease (more than 25 percent) in the diastolic reading of the occluded side. The following cases of complete occlusion of the carotid are of special interest.

CASE 12

This man had a traumatic carotid cavernous fistula. The right carotid artery was tied off in the neck. A significant decrease of 33 percent was found on the side of the occlusion.

CASE 13

This man was admitted with left hemiparesis of recent onset. Ophthalmodynamometer readings were 80 gm. in the right eye and 53 gm. in the left eye, a decrease of 34 percent on the left. Subsequent arteriography revealed complete occlusion of the left internal carotid and partial occlusion of the right internal carotid artery. The preoperative findings were substantiated at operation and endarterectomies were done. Ophthalmodynamometry was helpful in this case in localization of a lesion opposite to the clinically suspected side. This in turn helped to demonstrate the involvement of multiple vessels in this patient, unsuspected by physical examination or history.

CASE 14

This man was admitted to the Neurologic Service with left hemiparesis of mild degree and gradual onset, and weakness of the left side of the face. Occlusive digital compression of the common carotid on each side created no additional symptoms. Ophthalmodynamometry revealed a reading on the right of 60 gm. and on the left of 70 gm. This was a reduction of 14 percent on the right. Arteriography revealed complete occlusion of the right internal carotid artery with a normal left internal carotid artery. At operation, right internal carotid occlusion was confirmed. The absence of any large

TABLE 3

COMPARATIVE RETINAL ARTERY DIASTOLIC PRESSURES WITH PARTIAL CAROTID OCCLUSION

Case	Diastolic Readings (in gm.)		Decrease (percent)	Partial Occlusion Shown by	
	Open	Occluded		Angiography	Operation
21	93	88	5	?	Yes
22	80	78	2	Yes	Yes
23	65	65	—	No	Yes?
24*	65	61	6	Yes	Yes

* Complete occlusion on one side, partial occlusion on other side.

significant difference in ophthalmodynamometer readings and a negative carotid compression test suggest a good collateral circulation in this patient.

CASE 16

This man was admitted with a clinical diagnosis of complete occlusion of the left internal carotid artery, and this was confirmed at operation. Endarterectomy was performed on the left internal carotid artery. It was the impression of the surgeon that the obstruction was removed and the artery was patent after endarterectomy. A subsequent arteriogram on the right showed narrowing of the right internal carotid artery. Ophthalmodynamometry was performed at this time to compare the pressures of the retinal arteries. To the surprise of everyone, the reading for the right central retinal artery was 65 gm. and for the left central retinal artery 42 gm. This raised the question as to whether the left carotid artery, which had been opened only several weeks ago, was still patent. Furthermore, this raised the question as to whether the right internal carotid artery should be operated upon for narrowing (indicated on angiography) if the left internal carotid had become occluded since operation. Since it was the general impression that the left internal carotid artery must be patent despite the ophthalmodynamometer readings, operation on the right internal carotid was performed. However, when the right common carotid was clamped for a portion of the operation, the patient became convulsive, demonstrating that the left internal carotid had probably become occluded since the previous endarterectomy. In this case, ophthalmodynamometry gave valuable information on the postoperative status of one internal carotid system at a time when operation was planned on the other carotid system.

CASE 18

This man had hypertension and one-sided weakness. On carotid arteriography definite carotid occlusion was demonstrated and removed at operation. No consistent ophthalmodynamometer readings could be made, since the readings varied from 40 gm. to about 70 gm. on the same eye of either side with successive readings. The inconsistent readings may have been related to his labile hypertension.

CASE 19

This right-handed patient was admitted to the General Medical Service with aphasia, finger agnosia, and right homonymous hemianopsia. Ophthalmodynamometer readings were 85 gm. on the right and 60 gm. on the left, a decrease of 29 percent. Bilateral arteriograms revealed complete block of the left internal carotid artery. Endarterectomy was successfully performed on the left internal carotid artery, although symptoms were not greatly improved postoperatively.

Table 3 shows the comparative diastolic pressures in the retinal arteries of patients with partial carotid occlusion. The percentage decrease is much less, ranging from no significant difference to six percent.

CASE 22

This man had a severe right hemiplegia of five days' duration. Ophthalmodynamometry revealed an insignificant difference in pressures of the central retinal arteries, 80 gm. on the right and 78 gm. on the left, a difference of about two per cent. Carotid angiograms revealed a narrowing of the left internal carotid artery without complete occlusion. A left cervical sympathetic block resulted in satisfactory return of motor function in the lower extremity and partial return of function in the upper extremity. A left superior cervical ganglionectomy and left internal carotid endarterectomy were performed with gradual but gratifying improvement.

CASE 24

This man was demonstrated by carotid arteriography and at operation to have complete carotid occlusion on one side and partial interr.' carotid occlusion on the other side. Ophthalmodynamometer readings showed a six percent decrease on the side of the complete occlusion. There did not appear to be a great decrease in the level of the diastolic readings, as might be expected with complete carotid obstruction on one side and partial carotid obstruction on the other side.

TABLE 4

RETINAL ARTERY DIASTOLIC PRESSURES WITH QUESTIONABLE OCCLUSION OF CAROTID SYSTEM

Case	Diastolic Readings (in gm.)		Decrease (percent)	Clinical Impression
	Open	Occluded		
25	62	52	12	Probable occlusion
26	60	47	21	Probable postoperative occlusion
27	55	17	69	Intracranial carotid occlusion

In Table 4 is shown comparison of diastolic pressures in the retinal arteries of patients with questionable occlusion of the carotid system. Definite occlusion of the carotid arteries was not proved either by angiography or by operation at the time of the ophthalmodynamometer readings.

CASE 27

This woman had a vague history of syncope. The left common carotid artery had only about one-fifth as much pulsation as the right common carotid. Occlusion of the left common carotid caused no difficulty, but occlusion of the right common carotid caused sensations of passing out and could not be tolerated longer than 20 seconds. Clinically, the impression was that she had stenosis of the left commmon carotid artery. Ophthalmodynamometry revealed a reading in the right eye of 55 gm. and in the left eye of 17 gm., a large difference of 69 percent. Bilateral carotid angiograms showed a normal carotid artery on the right but could not demonstrate the left carotid. At exploration no apparent obstruction was found in the common carotid and the internal carotid on the left at operation. Ophthalmodynamometry was repeated after operation with approximately the same percentage decrease in the diastolic pressure of the central retinal artery on the left. Occlusion in the intracranial portion of the left internal carotid artery, or perhaps in the ophthalmic artery, was suspected to explain the ophthalmodynamometer findings.

SUMMARY

By means of ophthalmodynamometry, differences in the pressures of the central retinal arteries can be detected with a reasonable degree of accuracy and consistency. Six of nine patients with occlusion of the internal carotid artery on one side showed a 25 percent or greater lowering of pressure in the retinal artery on the side of the occlusion. However, two of the nine patients with complete unilateral carotid occlusion showed a decrease in the pressure of the central retinal artery of only 14 and 15 percent ipsilateral to the obstruction. These small differences in pressures of the essential retinal arteries were probably related to excellent collateral circulation. In a group of patients with apparently normal patent carotid systems, there was usually no appreciable difference between pressures of the central retinal arteries, with the exception of two patients who had differences of 16 percent. However, in no patient presumed to have patent carotid vessels did the ophthalmodynamometer readings of the diastolic pressures differ by more than 16 percent between the two eyes. In three patients after carotid operations, the ophthalmodynamometer continued to show a decrease in pressures of the central retinal artery on the operated side suggestive of partial unrelieved carotid occlusion. The ophthalmodynamometer would seem to be an early, safe means of detecting re-occlusion of a carotid artery after vascular surgery. Ophthalmodynamometry is a simple, safe and usually accurate method of estimating the relative pressures between the two internal carotid systems.

3503 Prytania.

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MUSCLE TRANSPLANTS TO SCLERA IN ANIMALS*

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In 1956, Burnside[1] advanced the medial one half of the inferior oblique muscle to the sclera over the macular area in the eyes of three monkeys in an attempt to increase the blood supply to the retina in that area. In one of these animals the retinas had been previously sunburned by direct exposure to sunlight for four minutes. His histologic sections suggested to him that an increased blood supply to the macula was produced by this operative maneuver. He[2] further studied the blood volume of the area using radioactive tracer methods with phosphorous isotopes in a series of dogs; but found that this method was not a reliable way of measuring the blood vo'ume of the area. Encouraged by his histologic findings, however, and aware of the seriousness of the problem of senile macular degeneration, Burnside[2] performed this operation on 28 patients with senile macular degeneration with the remarkable result of a definite improvement in vision in 27 of them.

Stimulated by his findings, we have performed muscle transplants to the sclera in the macular area in 20 rabbit and two cat eyes. Six of the rabbit eyes had previously had the retina coagulated with the light coagulator of Meyer-Schwickerath.[3] In three of these eyes, extraocular muscle was transplanted to the sclera at the site of the burn. The superior rectus muscle was used for the transplant. The eyes were sectioned at varying intervals from three days to three months after surgery.

Although new granulation tissue was found in the site of the anastomosis on the surface of the sclera, in no case could we find definite ingrowth of vessels into the sclera suggesting increased blood supply to the choroid. Those animals treated with the light coagulator and muscle transplant showed no increased evidence of repair when compared to other areas burned with the light coagulator without muscle having been transplanted beneath them (figs. 1 to 5).

COMMENT

Senile macular degeneration is an extremely important clinical problem to which only a relatively small amount of research has been directed. It has been presumed in the past that this condition is due to a de-

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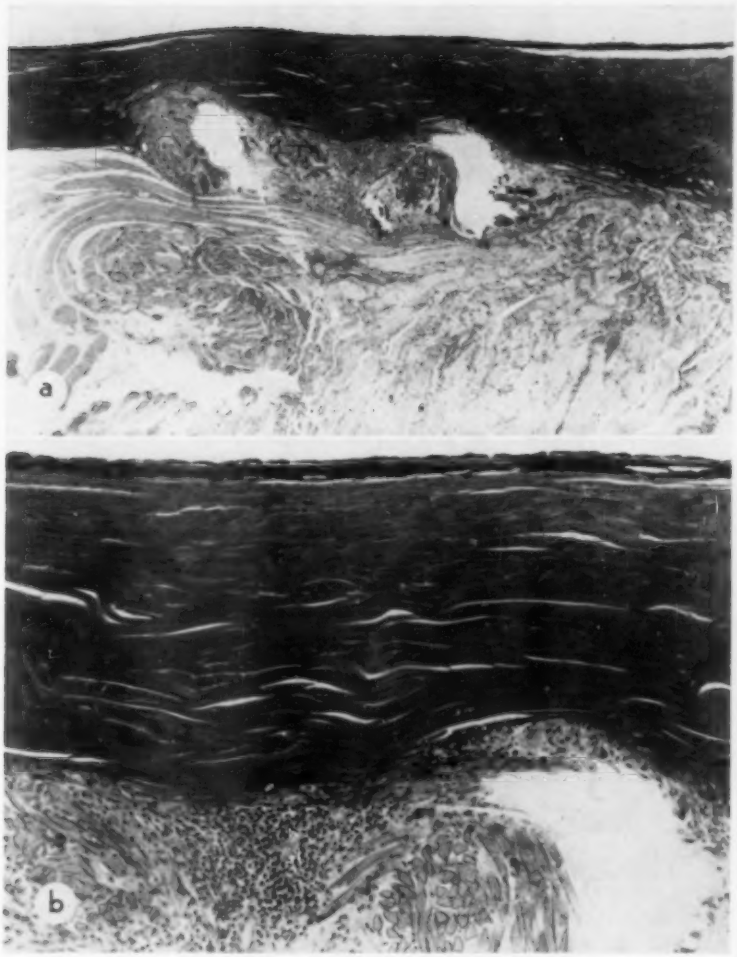

Fig. 1 (Westsmith, Demorest and Flocks). Rabbit 8. (a) Site of muscle anastomosis to the sclera. Bits of suture material can be seen. The choroid appears normal. The normal retina is artificially detached. (b) High-power view same eye. The sclera is not invaded by new vessels.

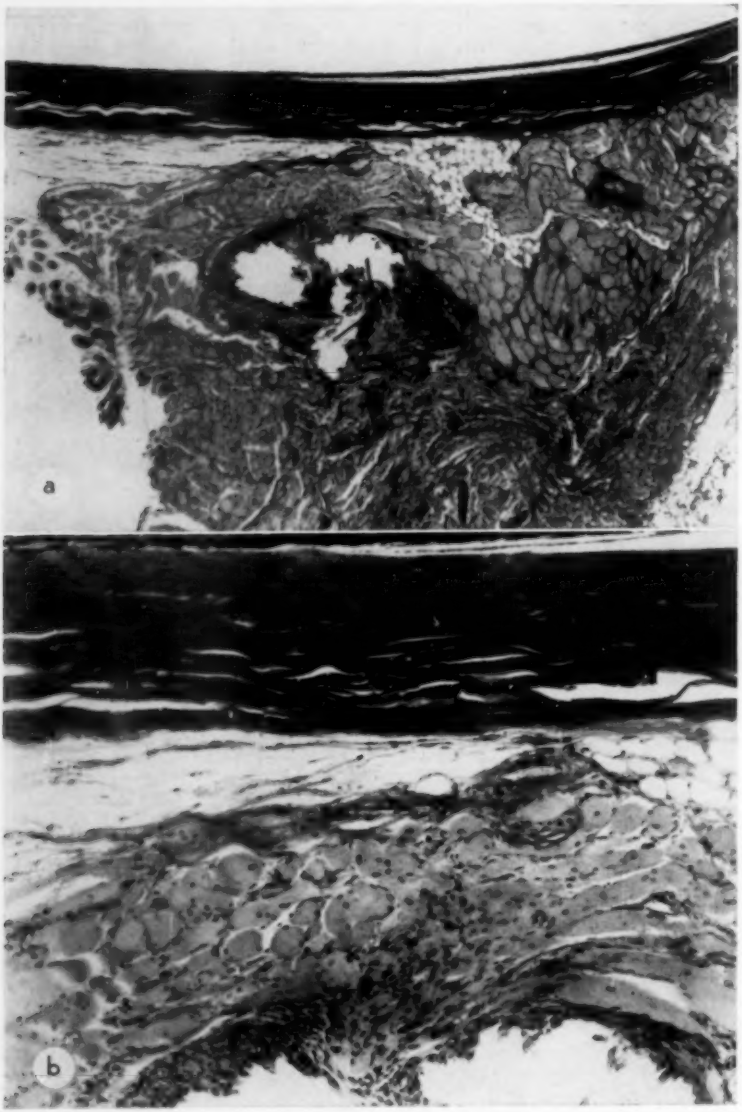

Fig. 2 (Westsmith, Demorest and Flocks). Rabbit 9. (a) Site of muscle anastomosis. The retina is artificially detached. (b) High-power view, same eye. The sclera and choroid appear unaffected.

Fig. 3 (Westsmith, Demorest and Flocks). Rabbit 6. The suture material and muscle are well into the wall of the sclera, but no new growth of blood vessels to the choroid is seen.

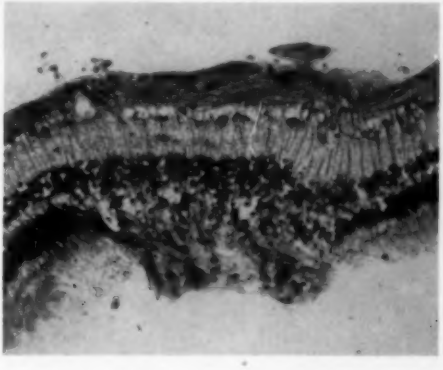

Fig. 4 (Westsmith, Demorest and Flocks). Rabbit 13. An area of rabbit retina which has been treated with the light coagulator. The disturbance is marked in the deepest layers of the retina near the pigment epithelium.

crease in the blood supply to the area, but this is by no means definitely proven. Our results indicate that transplantation of an extraocular muscle to the sclera in the macular area does not increase the blood supply to the sclera in that area. From our findings in rabbits and cats, we are at a loss to explain the definite visual improvement achieved by Burnside in 27 of 28 patients following inferior oblique advancement. We can speculate that perhaps the muscle transplants furnished needed support to the posterior pole of the eye in the same way that Borley[4] has improved macular vision in high myopes by means of scleral transplants; or that the improvement was due to some other reason. We shall be extremely interested in the results of Burnside's continuing series of patients treated surgically for senile macular degeneration.

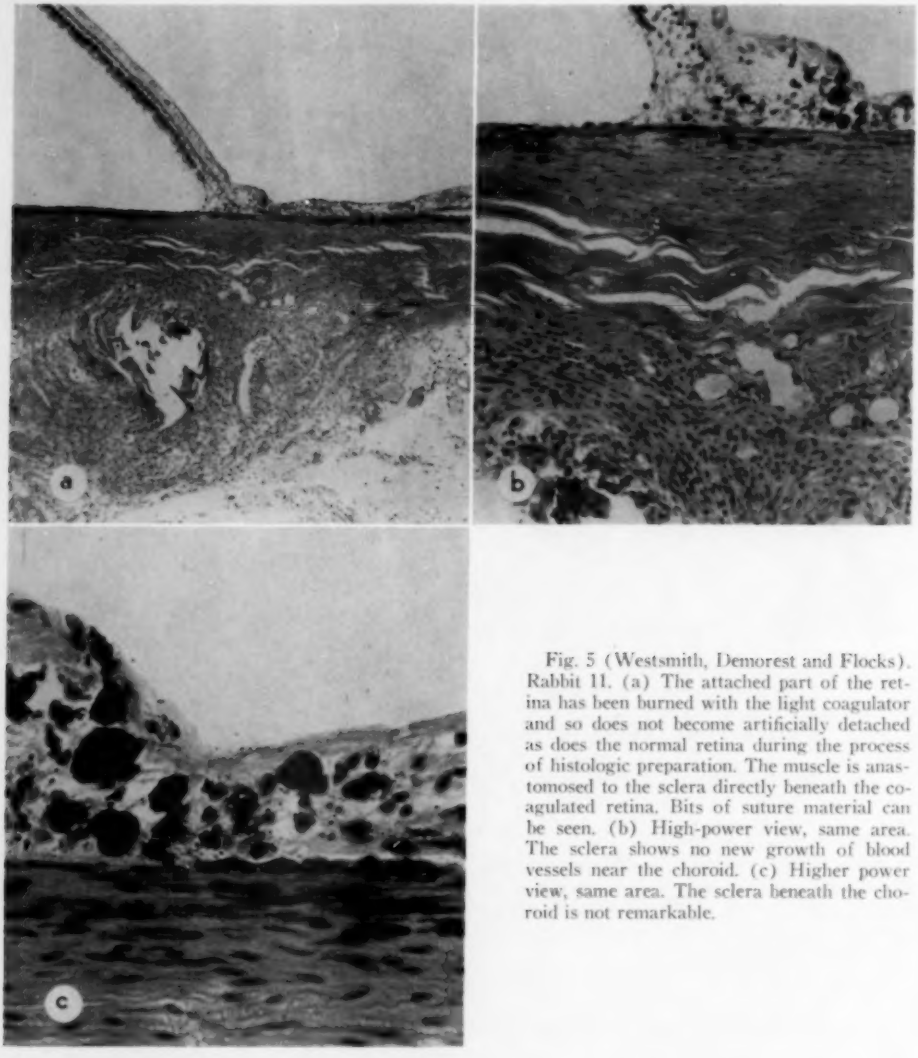

Fig. 5 (Westsmith, Demorest and Flocks). Rabbit 11. (a) The attached part of the retina has been burned with the light coagulator and so does not become artificially detached as does the normal retina during the process of histologic preparation. The muscle is anastomosed to the sclera directly beneath the coagulated retina. Bits of suture material can be seen. (b) High-power view, same area. The sclera shows no new growth of blood vessels near the choroid. (c) Higher power view, same area. The sclera beneath the choroid is not remarkable.

Summary

Extraocular muscle transplants to the sclera in the region of the macula do not produce histologic evidence of increased blood supply to the choroid or retina in rabbits.

Clay and Webster Streets (15).

Acknowledgment

We wish to thank Dr. Dohrmann K. Pischel and Dr. Bayard Colyear for producing light coagulation of the rabbit retinas with their apparatus.

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OPHTHALMOTROPIC DETERMINATION OF THE COMBINED ACTIONS OF THE EXTRAOCULAR MUSCLES*

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Introduction

The information presented in this report was gathered to be used in the design of a motorized ophthalmotrope. The motorized ophthalmotrope is to be designed to reproduce actual eye movements as closely as possible. In order to be able to design such an ophthalmotrope it was necessary to determine the amount of muscle action required by each of the six extraocular muscles to get the eye into all direction of regard. It was felt that the data obtained might be of value to other investigators studying the action of the extraocular muscles. The data were obtained by specifying eye positions in accordance with Helmholtz's system of co-ordinates,[1] but the values can very easily be transposed to any other system. An example of such a transposition is given in the text.

Apparatus

The apparatus consisted of an ophthalmotrope containing a miniature projection system and a plane projection screen located 10 inches in front of the center of rotation of the ophthalmotrope. The two units were mounted on a rigid base common to both components. Views of the ophthalmotrope and the projection screen are shown in Figures 1, 2 and 3.

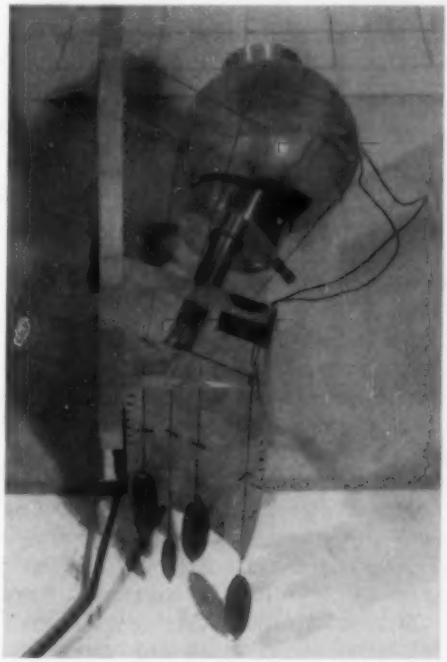

Fig. 1 (Knoll). Ophthalmotrope representing the right eye as seen from above.

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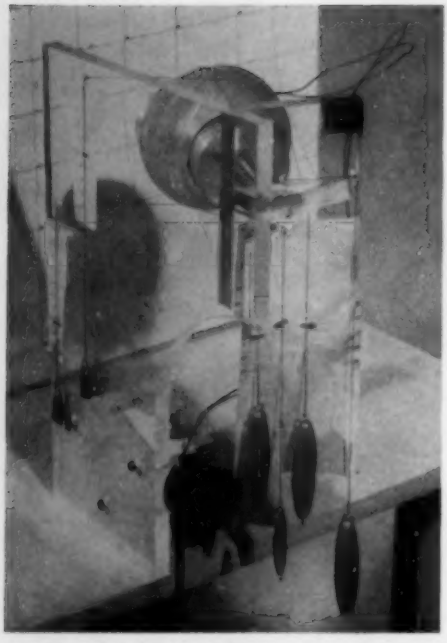

Fig. 2 (Knoll). Ophthalmotrope representing the right eye seen from a posterior medial aspect. The muscle weights and scales can be seen. The projection screen with the coordinates of azimuth and elevation are seen behind the ophthalmotrope.

The globe of the ophthalmotrope was made from a hollow plastic sphere 101.6 mm. in diameter. This sphere rotated on a cylindrical cup made of aluminum tubing mounted on a brass rod attached to the "apex" of the orbit. The cup can be connected to a vacuum line to hold the globe fixed in a given position. The vacuum fitting can be easily seen in Figure 3. Rotation of the globe on this cup placed the center of rotation of the globe at its own center of curvature, a condition which does not exactly duplicate the circumstances in the living eye. This and the fact that the center of rotation is not a fixed point in the living eye are two approximations which had to be made in the ophthalmotrope. These are not serious approximations, however, and will not invalidate the data.

Other approximations involve the "muscles" used. Silk threads were used to represent the central axis of each muscle. Origins and insertions were used which would represent the centers of each of the six muscles. No attempt was made to duplicate the effects of the muscle sheath attachments to each other. Furthermore, no attempt was made to duplicate the effects of the other orbital contents. These effects are very complicated and would be difficult to reproduce.

The four recti muscles were attached as follows: The insertions were located in reference to the "limbus," a circle having a diameter of 48 mm. centered on the sagittal diameter of the eye. Medial rectus muscle insertion: 22 millimeters from the limbus on the 0-degree meridian. Inferior rectus muscle insertion: 26 mm. from the limbus on the 270-degree meridian. Lateral rectus muscle insertion: 28 mm. from the limbus on the 180-degree meridian. Superior rectus muscle insertion: 33 mm. from the limbus on the 90-degree meridian. The origins of the four rectus muscles were located on a circle having a diameter of 32 mm. centered about the brass support rod at the "optic foramen."

The insertion of the superior oblique muscle was on 90-degree meridian 19 mm. behind the equator. Its "origin" was on the medial wall of the orbit such that its course made an angle of 55 degrees 21 minutes with the wall of the orbit. In its course it passed under the superior rectus muscle. The insertion of the inferior oblique muscle was 12

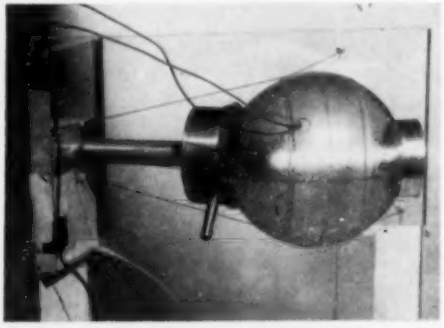

Fig. 3 (Knoll). Ophthalmotrope representing the right eye seen from the lateral aspect.

mm. below the 180-degree meridian, 30 mm. behind the equator. Its origin was on the medial wall of the orbit such that its course made an angle of 50 degrees 27 minutes with the wall of the orbit.

The medial wall of the orbit was represented by a vertical sheet of plastic oriented perpendicular to the projection screen. The center of the globe was located 81.8 mm. lateral to the wall. The center of the globe was located 135 mm. from the plane of the optic foramen.

The muscle strings passed through small holes in the plastic sheets and were attached to lead weights. Each of the muscle strings carried an indicator whose position could be read against a vertical mm. scale. These weights and scales can be seen in Figure 2.

The miniature projector mounted within the globe served to represent the retinal vertical and horizontal on the projection screen. A seven watt bulb was located behind a thin sheet of metal which had a small cross punched out of its center. The cross was imaged on the screen by means of a plano convex lens.

The projection screen carried a grid representing a gnomonic projection of the coordinates of azimuth and elevation. The muscle lengths were plotted for each ten degrees of azimuth and elevation from minus 40 degrees (left) to plus 40 degrees (right) azimuth and from plus 40 degrees (up) to minus 40 degrees (down) elevation. At each position the appropriate amount of torsion was introduced in accordance with Listing's law.[2]

The data were taken as though the eye was fixed at a certain value of elevation and changed its position in azimuth. This would correspond to having the eye sweep horizontal lines parallel to the frontal plane at optical infinity.

Results

The data are presented in paired graphs representing the paired muscles. Since the model represents the right eye, minus azimuth values represent movements toward the nose, positive azimuth values represent movements toward the temple. The points on these curves represent the actual values measured. The curves have been fitted by eye.

In Figure 4 may be seen the results for the horizontal recti. As expected, the change

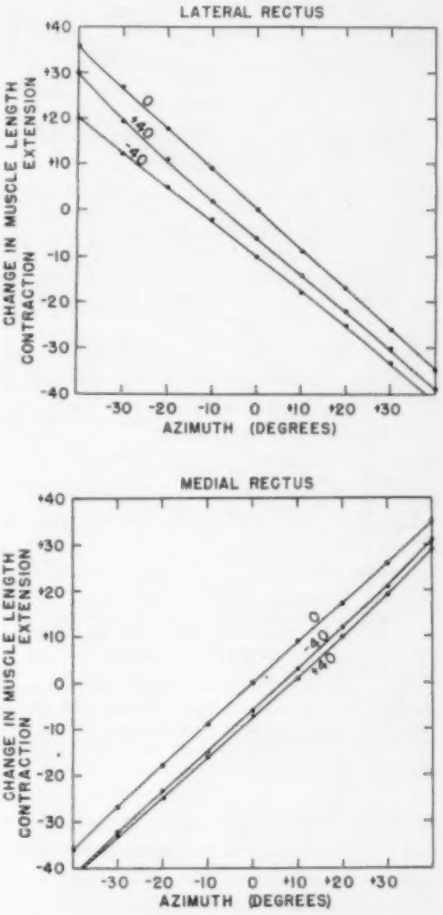

Fig. 4 (Knoll). Change in muscle length (mm.) for the medial and lateral recti muscle of the right eye. Minus azimuth represents nasal direction. The numbers given above the curves represent the elevation angles. The points 10, 20, 30 degrees elevation have been omitted since they all fall between the values shown.

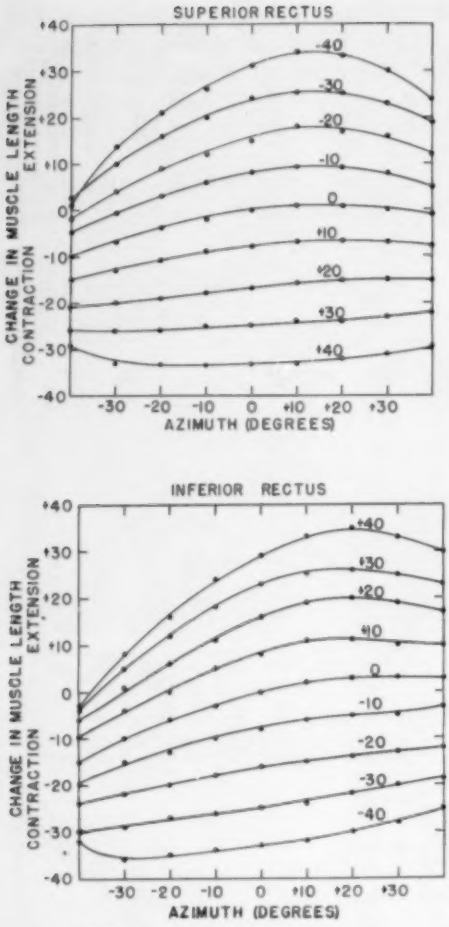

Fig. 5 (Knoll). Change in muscle length (mm.) for the superior and inferior recti muscles of the right eye. Minus azimuth represents nasal direction. The numbers given above each curve represent the elevation angles.

in muscle length is for all practical purposes a straight line function. The muscle lengths change very little as the eye is elevated or depressed, hence the values between the extremes have been left off the graphs.

The results for the vertical recti are shown in Figure 5. It will be noted that these muscles do have changes in length which are not reciprocally related. For example, when the eye is elevated (plus 40), the changes in the superior rectus are minimal, but fairly large changes take place in the length of the inferior rectus. The reverse is true when the eye is depressed. This difference is explained by the fact that the contracted muscle has its insertion close to the axis of rotation, whereas the extended muscle has its insertion at some distance in front of the axis of rotation and hence will swing

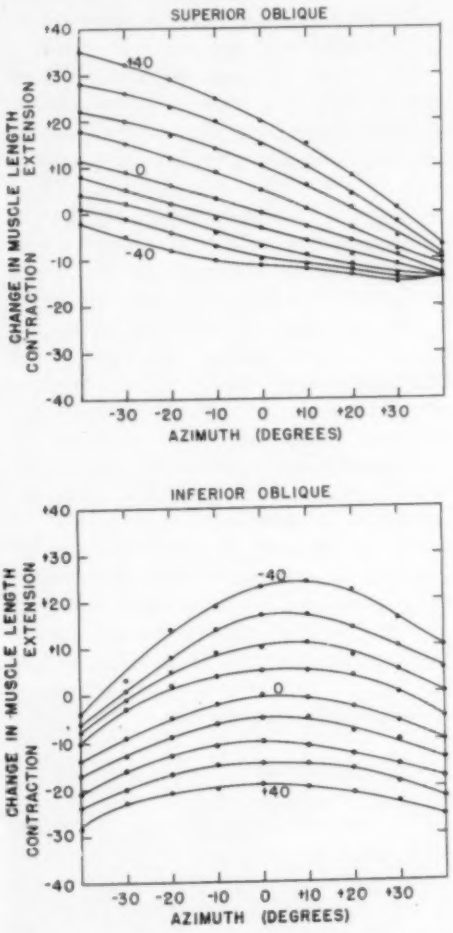

Fig. 6 (Knoll). Change in muscle length (mm.) for the superior and inferior oblique muscles of the right eye. Minus azimuth represents nasal direction. The elevation angles are given only for 0 and 40 degree elevation. The other elevation curves can be found between these.

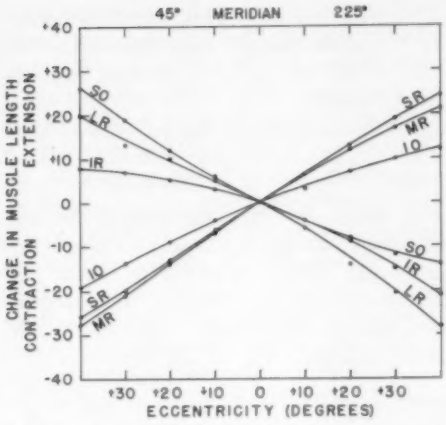

Fig. 7 (Knoll). Change in muscle length (mm.) for all six muscles of the right eye as it moves along the 45-225 degree (up, nasalward—down, temporalward) meridian. Eccentricity is the angular displacement of the line of sight from the primary position along the meridian.

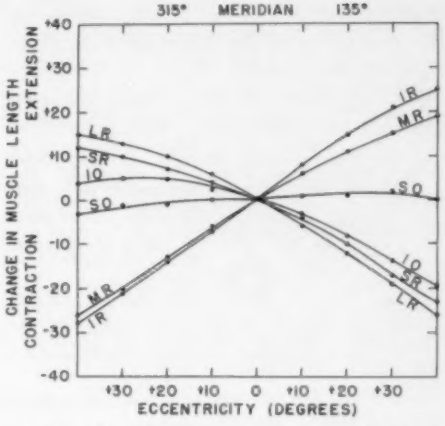

Fig. 8 (Knoll). Change in muscle length (mm.) for all six muscles of the right eye, as it moves along the 135-315 degree (up, temporalward—down, nasalward) meridian. Eccentricity is the angular displacement of the line of sight from the primary position along the meridian.

through an arc which has its maximum radius when the eye is looking straight ahead and its minimum radius upon looking nasally.

The results for the oblique muscles are shown in Figure 6. Again, as expected, the changes in muscle length are quite different for the two obliques. Note that most of the action of the superior oblique is in the elevated position in extension. For the inferior oblique the action is fairly well divided between elevation and depression, but the greatest changes take place in muscle extension. It is also of interest to note that the superior oblique has its maximum vertical activity nasally, whereas the maximum vertical activity of the inferior oblique appears to be in the straight ahead position. These positions correspond to those positions for which the insertions are displaced maximally from the axis of rotation of the globe.

The extension of these data can be demonstrated by choosing eye movements along some other system of coordinates. For example, the changes in muscle length may be desired as the eye passes from its primary position along oblique meridians. Such data have been extracted from Figures 4, 5 and 6 to show the changes in muscle length as the right eye moves along the 45 to 225 degres (up, nasalward—down, temporalward), and 135 to 315 degrees (up, temporalward—down, nasalward) meridians. These data are plotted in Figures 7 and 8. Here it is interesting to note that all the muscle functions approximate straight lines when movement within twenty degrees of straight ahead is considered.

Summary

An accurately scaled ophthalmotrope has been constructed to determine the muscle actions for all directions of regard of the right eye. These data have been presented in the form of graphs. The data are given in terms of angles of azimuth and elevation.

A transposition to angles of meridional direction and eccentricity is given also. The following generalizations emerge upon examination of the graphs:

1. The lateral muscles change very slightly in length with elevation and depression of the line of sight.

2. The vertical muscles, when extended,

undergo greater changes in length with lateral excursions of the line of sight than they do when contracted.

3. The actions of the obliques are quite different from each other:

a. The greatest changes in the length of the superior oblique muscle during vertical excursions take place when the line of sight is directed nasally whereas,

b. The changes in the inferior oblique, during vertical excursions of the line of sight, are fairly uniform for nasal and temporal positions. Maximum changes take place when the line of sight is directed straight ahead.

c. The action of the superior oblique muscle is largely extension.

4. When the data are plotted for eye movements along meridians, the changes approximate linear relationships when the movements are restricted to displacement less than twenty degrees from the straight ahead position.

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Discussion

Jay M. Enoch (St. Louis): May I suggest that the ophthalmotrope you have described would be very useful to demonstrate the changes in muscle action which would result with changes or variations in muscle insertions.

Hermann M. Burian (Iowa City): Were the "muscles" of the ophthalmotrope that you have described arranged in such a way that their functional insertions were always realized?

Henry A. Knoll: Dr. Enoch's suggestion is a very pertinent one and as a matter of fact this concept bothered us a great deal when it came to placing the insertion for the superior oblique. However, a value was chosen and, of course, other insertions could now be tried to see their effects. I am hoping to be able to have a mathematician construct a mathematical model which can then be manipulated more readily than the ophthalmotrope in any of the many parameters.

In answer to Dr. Burian's question, I can only say that the muscle threads were allowed to slip and slide over each other and over the surface of the globe as the geometry of the various eye positions dictated. For the model at least, these were the functional insertions.

CORNEAL HEALING

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In order to evaluate adequately corneal healing one must consider the embryology, anatomy, chemistry, physiology, metabolism, and vascular and nerve supply.

Embryology

Hagedoorn believes and suggests that the endothelium which is again said to be of the first mesodermal element of the cornea grows in from the periphery behind the primitive ectodermal lamina, while at a later date the mesodermal stroma grows in edgewise between the lamina and the epithelium. In which case, then, we would believe that the cornea is probably a mesodermal tissue except for Bowman's membrane and the surface epithelial cells.

Anatomy

Anatomically the cornea is a clear transparent multilayered membrane, approximately 0.5 mm. thick centrally and 0.6 mm.

thick peripherally, having a convex-concave shape with a diameter of approximately 11.5 mm. and with a smooth and brilliant surface. It fits with a bevel margin into the anterior scleral foramen. In order that this thick margin may accommodate itself to the beveled edge of the sclera, the inner margin of the cornea is rounded off in a deep groove, the sulcus circularis corneae, which fits into the internal scleral sulcus.

The cornea proper is composed of five layers: (1) epithelium, (2) Bowman's membrane, (3) substantia propria, (4) Descemet's membrane, and (5) endothelium.

The epithelium of the cornea is continuous with that of the conjunctiva, and the stroma of the cornea is continuous with that of the sclera while Bowman's membrane, Descemet's membrane, and the endothelium are co-extensive with the cornea. The cornea consists of two zones; (1) the cornea proper and, (2) the limbus or peripheral portion; and these portions do differ histologically and also in that vessels carrying blood and lymph are present at the limbus.

Epithelium

The corneal epithelium is contiguous with the bulbar conjunctiva which is attached rather loosely to the anterior surface of the cornea, and is attached firmly only in a narrow often ridgelike strip known as the annular conjunctiva. The epithelium is five or six cells thick, morphologically unlike other stratified squamous mucous membranes (including the conjunctiva) in at least two important respects: its outer surface maintains a mirrorlike smoothness and its inner surface is relatively loosely bound to the underlying tissue. It is notably homogeneous, containing none of the goblet or other specialized cells that are usually present in mucous membrane. There are three types of cells present in the epithelium:

1. The basal cells are deeply situated and rest on Bowman's membrane. These cells have round heads and are flat at their base; they are large cuboidal or low columnar in type. They also have a clear cytoplasm and a round or oval nucleus and a diplosome in the apical part of the cell body.

2. The wing cells are of polyhedral shape and are arranged in three layers. The nuclei are oval and parallel to the surface.

3. The outer surface is composed of quite smooth and large squamous cells. In successive corneal layers from the deepest to the most superficial, the cells become increasingly flattened. The cells in the corneal epithelium are linked with one another by intercellular protoplasmic bridges known as "prickle" cells. The cells of Langerhans appear between the corneal cells. These are wandering leukocytes that have migrated from the peripheral blood vessels and appear as irregular branched elements.

Recently Teng and Katzin have made extensive studies of the basement membrane of the corneal epithelium stating that it is a continuous membrane between the epithelial layer and Bowman's membrane which extends to the limbus and beyond it to the bulbar conjunctiva. Anteriorly, it follows closely the curve of the posterior surface of the basal layers of the epithelium. Posteriorly, it rests directly on Bowman's membrane. The thickness varies with individuals and with pathologic change, and is approximately one-third the thickness of Bowman's membrane.

Of course the corneal epithelium has tremendous powers of regeneration, and it may even slide a little bit on the basement membrane. This can be demonstrated by rubbing the eyelids and then looking at the cornea with the biomicroscope. Small defects or mild trauma to the cornea heal rapidly by a gliding and flattening motion of the adjacent cells.

Mitoses begins in the basal layer of cells and may be found a considerable distance from the site of injury. In fact, mitoses may occur in this layer under normal conditions, that is, when the epithelium has not been injured.

Recent experiments according to Cogan

in which butyl alcohol was found to be an effective loosening agent suggests that the substance holding the epithelium in place is a lipid, but there is also evidence that the epithelium-stroma cohesion is loosened by enzymatic inhibition (iodoacetate; fluoride), suggesting that it is dependent on an energetic process. Histamine also appears to be a specific epithelial loosening agent. Cellular cohesion is a highly complex system and its loss can be produced experimentally by proteolytic enzymes, but no appreciable effect occurs as a result of interference with respiratory processes in the cornea.

The individual cells are presumed to advance forward as they mature in the epithelium, and the most superficial layers become progressively eosinophilic (or less basophilic). Ultimately the cells are shed from the anterior surface, and occasionally one sees turgid nucleated cells on the surface of the cornea, suggesting that the individual cells swell up just prior to desquamation.

Bowman's membrane

Bowman's membrane is a hyaline lamina clear and uniform, ranging between 8.0 and 12 microns in thickness. It lies between the epithelium and the stroma proper and is analogous to other basement membranes. It is found only in primates. It is acellular, and its origin is not clearly evident, although it is commonly supposed to be derived from the most anterior portion of the stroma. Except for being somewhat less eosinophilic, its staining reactions are similar to those of the corneal stroma; it does not take the elastic tissue stain. It covers the entire cornea and ends abruptly in the conjunctival stroma. The function of Bowman's membrane is open to speculation, but it seems most likely to assist in the maintenance of a generally smooth anterior surface of the cornea, and probably forms a partial barrier to the penetration of cells into the cornea. This is strikingly seen when tumors grow on the surface of the cornea and appear to be prevented from invading the cornea by the presence of Bowman's membrane. There are, however, perforations of Bowman's membrane for the passage of nerves from the stroma into the epithelium, and these channels are also used by wandering cells which sometimes form clusters at the outer end of the canal, accounting perhaps for the punctate distribution of superficial infiltrates in certain corneal diseases.

Bowman's membrane does not regenerate when destroyed, although it is quite resistant to trauma and infection. It has many properties similar to the corneal stroma.

Corneal stroma

The corneal substantia propria comprises about 90 percent of the entire thickness of the cornea and is a transparent modified connective tissue structure, made up of lamellae and cells. It is replete with intercellular laminas and has a paucity of cells, and an absence of blood and lymph vessels. The laminas are compact, eosinophilic bands and number about 60; these consist of broad bands of fibrils 1.3 to 2.5 microns thick which traverse the entire length of the cornea as meridional curves, parallel to the surface.

Cogan has attempted to differentiate the fibers into a superficial layer forming an interlacing network with a narrow, circular band at the periphery. The middle layer is composed of fibers radiating out fanwise in the direction of the four rectus muscles. The deepest layers form an interlacing network centrally but a broad circular band eccentrically which extends to the limbus. Between the lamellae, the corneal corpuscles can be demonstrated. Cogan also states that there is no positive evidence, contrary to what one commonly reads, of a cement substance holding the laminas together. In the anterior half of the cornea they are more compactly interdigitated than they are in the posterior half of the cornea, offer greater resistance to spread of India ink, and on rupturing are more apt to form fissural cracks.

Laterally the fibers of the cornea extend into those of the sclera without any abrupt transition such as one might expect if there were a histologic counterpart of the optical difference between the transparent cornea and the opaque sclera. In the usual well-prepared cross section of the cornea one gains the impression that all the laminas course parallel to the plane of the section, and this is true paradoxically whether the sections are cut horizontally or vertically. But when the cornea is slightly swollen, the laminas running in opposite direction may be seen in approximately alternate layers.

The cells are compressed between the laminas and appear to be ordinary connective tissue cells; but in horizontal preparations they may be seen to be cells of a respectable size forming an extensive syncytium between the laminas. In swollen corneas or in tissue cultures the cells may be seen to have spindle-shaped nuclei and abundant cytoplasmic processes identical with those of connective tissues elsewhere. These have spindle-shaped nuclei and abundant cytoplasmic processes, as is typical of connective tissue generally. These cells form and maintain the collagenous laminas and act as phagocytes. The nuclei of the corneal cells are round or elliptical in the newborn, but are irregular in the adult. Krawicz, quoting from Thomas, stated that these corneal cells are functionally similar to the basic components of the reticulo-endothelial system. He studied their colloidopexic properties by means of silver compounds. They correspond to prohistiocytes and have a distinct storing property for silver. Ciotola also, according to Thomas, has performed experiments to indicate the presence of elements of the reticulo-endothelial system in the parenchyma of the cornea. He found cellular elements that absorbed vital dyes during iontophoresis.

Besides the fibroblasts the stroma also contains a number of lymphoid wandering cells which migrate from the blood vessels of the corneal limbus. When inflammation is present, the tissue is infiltrated with enormous numbers of heterophil leukocytes and lymphoid cells which penetrate between the lamellae and become arranged in typical fusiform rows.

Cogan in his ultramicroscopic structure analysis of the stroma states:

"From examination of the individual stromal fibers with polarized light it is possible to say that the corneal fibers, like those of collagen elsewhere including the sclera, have a crystalline structure that is amenable to quantitative measurements, and the crystals are oriented parallel with the long axis of the fibers (positive uniaxial birefringence). Moreover, the Maltese cross that is produced when the whole cornea is examined between crossed polarizers suggests that the fibers are arranged radially to the vertex of the cornea. This regularity of the micelles is said to enhance in some way the transparency of the cornea in the sagittal direction. It has also been suggested that the appearance of a cross may be attributable to the oblique incidence of the light falling on the anisotropic corneal lamellas. Stretching of the cornea results in an incidence in the birefringence, and it has been suggested, but without substantial proof, that this increase on stretching accounts for the opacification of the cornea in glaucoma. In contrast to the stroma the epithelium shows very little birefringence except in its most superficial layers."

Descemet's membrane

Descemet's membrane is the posterior elastic lamina, a hyaline lamina, approximately 10 microns thick. It lays between the stroma and the endothelium. It is split up into many fine laminas at the periphery of the cornea to contribute to the scaffolding of the angle meshwork. It is readily separable from both the substantia propria and the endothelium, and is very resistant to inflammatory processes and can be isolated by mascerating the cornea in acid or alkali. Unlike Bowman's membrane, it readily reforms

after pathologic destruction; and when it is cut it gapes slightly and shows a tendency to roll up. It appears to be a homogeneous structure taking acid stains, most elastic tissue stains, and some of the stains for neutral polysaccharides. It appears to be loosely attached to the stroma since it may be easily separated by pus, blood, or often merely by the process of preparing the sections.

Occasionally circular bands of hyaline material may be found coursing within Descemet's membrane at its peripheral extremities, comprising the line seen by gonioscopy and known as Schwalbe's line. At the periphery it shows, in older eyes, excrescences known as Hassall-Henle warts. Cogan states that:

"The distinctive properties of Descemet's membrane are: (1) its property to 'take' elastic tissue stains; (2) the tendency for its cut edges to roll outward; (3) its imperviousness to invasion by blood vessels, cells, and other formed elements; and (4) its apparent resistance to pyolytic and autolytic processes in the body.

The functions of Descemet's membrane can be inferred from these properties. It is protection to the cornea from invasion of the inflammatory cells of the aqueous. The barrier that Descemet's membrane forms to the invasion by blood vessels is strikingly seen in transplantation experiments where no blood vessels get into the cornea, at least through its posterior surface, so long as this membrane is present and intact."

Endothelium

The endothelium is a single layer of flat hexagonal cells 5.0 microns high and 18 to 20 microns wide arranged along the inner surface of Descemet's membrane, and hence forms the lining of the posterior surface of the cornea. The cells constituting the membrane form a mosaic of pentagonal units and are somewhat larger and have more abundant cytoplasm than do most endothelial cells, and this as well as their origins has given rise to the suggestion that they are not true endothelial cells. Mitoses are also never seen in the normal endothelium, and its method and rate of growth are wholly obscure. Two of the distinctive characteristics of the endothelium from a morphologic point of view are (1) its marked fragility to mechanical injury and (2) its property of surrounding any objects with which it comes in contact.

The capacity of the endothelium to surround objects may result in incorporation of foreign matter (pigment, retrocorneal membranes, or small calcific particles) much as a bee incorporates foreign particles in its hive, and thereby avoids any disturbance of fluid exchange. Mitoses are never seen in the normal endothelium, and it is felt amitoses is a method of regeneration of corneal endothelium of the rabbit under normal conditions and that the mitotic cell division occurs under unusual circumtances only.

Limbus

Limbus is approximately one mm. wide, and includes the transition zone between the cornea and the conjunctiva, episclera and sclera, and it contains two layers, the epithelium and stroma. The structure of this region is different because Bowman's membrane stops short and Descemet's membrane merges in the formation of the meshwork of the angle of the iris, by passing over the trabeculas at the chamber angle. Both anatomically and physiologically in the limbus region, it has a close relationship to the cornea proper, because of its proximity and because many physiologic and pathologic changes in the cornea are influenced by or dependent on the blood vessels, lymphatics or aqueous veins, and nerves, situated in the limbus.

The cornea is devoid of blood vessels but the region of the limbus is plentifully supplied by the superficial and deep marginal plexus. The anterior ciliary arteries furnish branches to the sclera and also the anterior conjunctival arteries to the bulbar conjunctiva. These latter vessels anastomose with the posterior conjunctival arteries which are

branches of the medial palpebral vessels (small direct branch from the ophthalmic artery) and the lateral palpebral vessels (small branches from the lacrimal artery). These arteries form a rich anastomosis which ends in a series of arcades and give off fine branches to the extraordinarily delicate loop network at the margin of the cornea. These loops bend around to form vessels that lead into a delicate network similar to the arterial side of the arcades. These vascular loops may be very prominent and appear as concentric collaterals as described by Vail and Ascher. The aqueous veins are biomicroscopically visible pathways whose appearance simulates that of the blood vessels; they contain a clear colorless fluid or diluted blood, and are intercalated, probably via Schlemm's canal, between intra-ocular fluid on one side, and conjunctival and subconjunctival veins on the other. Anatomically, they are connected with, or form a part of the intrascleral mesh.

Corneal nerves

Undoubtedly the corneal nerves are derived essentially from the ciliary nerves, branches of which enter the sclera from the perichoroidal space a short distance behind the limbus. At the corneal margin they usually lose their myelin sheaths, and continue their course as transparent axis-cylinders, a few retaining them for some considerable distance; they run in the anterior two-thirds of the cornea, dividing usually dichotomously, rarely trichotomously, and very occasionally in a T-formation. Sometimes they show a bulbous formation at the points of division, or at intervals along their course, according to Duke-Elder. Schlemm described the nerves as originating in the ciliary nerves and consisting of both deep and superficial branches, terminating on the surface of the cornea. The ophthalmic branch of the trigeminal nerve, which is chiefly a sensory nerve with its center the gasserian ganglion, provides the corneal sensitivity through the ciliary nerves.

It is estimated that 60 to 80 nerve trunks penetrate the deep layers of the cornea, usually in the posterior two-thirds of this structure. After penetration they run a radial course and branch, as stated above, dichotomously or dichromously, the nerve trunks naturally diminishing in diameter as they progress in their course. The branching that takes place is usually at acute angles and at the bifurcation there is a web or dark-stained plate in most cases. There are also fine single nerve fibers that enter the cornea and follow the same course as the heavier nerve trunks. Thomas states that this communicating, interweaving and multilayered plexus of nerves is thought by some to follow the lacunar system of the cornea, and was termed the fundamental plexus by Martinez.

Escapini stated that the corneal nerves consist of three elements: (1) a sheath of Schwann; (2) the myelin sheath, and (3) the axis cylinder. Most of the fibers lose their myelin sheath about 0.5 millimeter from the limbus, before the branching occurs. The nerve endings, of course, penetrate Bowman's membrane and enter the epithelium. There are two special types of fibers shown, shorter fibers provided with end-plates ramifying in the superficial lamellae and the epithelium of the limbus, and a second type which shows no anastomoses or end-bulbs, but which ends in the substantia propria by tapering to a point. This evidence points to the fact that these nerves are nerves of pain, according to Duke-Elder.

Rexed and Rexed (1951) have shown that: "In nerve regeneration usually the proliferation of the Schwann cells bridges the gap and leads the regenerating neurites in the right direction. In all other peripheral nerves the neurites invariably grow out into the mass of proliferated Schwann sheaths, the so-called Bungners bands. Bethe (1903) considers that the Schwann cells form the new neurites, and Boeke (1935) considers them indispensable for the regeneration and growth of new neurites. The proliferation

of Schwann cells is, however, an independent reaction which has an identical course even in the absence of axons (Rexed, 1942)."

Corneal transparency

Quoting from Francis Heed Adler: "The normal cornea is transparent, and any change in this property seriously interferes with the clarity of the retinal image. The anatomical peculiarities of the corneal structure, such as the uniformity and regularity in the arrangement of the epithelial cells, the closely packed corneal lamellae running almost parallel to each other, the absence of blood vessels and other features, all contribute to the efficiency of the eye as an optical instrument. Because the individual corneal cells are living and therefore are constantly undergoing change and suffering replacement, the cornea cannot maintain an unvarying constancy in its transparency and optical properties."

There are many theories offered to explain the transparency of the cornea, the most recent and fruitful one was profounded by Maurice, a "lattice" theory which points out that in the cornea the small diameter, the regularity of the size and arrangement of the ultramicroscopic fibrils are the essential features which make this tissue transparent. The spaced fibrils behave as an infinite number of diffraction gratings. Because of their number and regularity of their arrangement, the intensity of the maxima is reinforced by the diffracted light and relatively little is lost by scatter.

The fibrils of the cornea have been shown to be very thin as compared to those which make up the sclera or even a corneal leukoma. The diameters of the corneal fibers are not very variable, whereas those of the opaque sclera or of a leukoma are so, demonstrated by the study of Schwarz. According to Maurice, for a tissue to be transparent, it is necessary that its fibrils be parallel, equal in diameter, and have their axes disposed in a lattice. We know that the corneal fibrils are fine, parallel and equal in diameter.

The X-ray defraction patterns of the cornea presented by Maurice are evidence that the fibrils are disposed in a lattice, thus permitting transparency. It is also suggested that one function of the interstitial mucoid may be in maintaining the orderliness of their arrangement. When this orderliness is disturbed by swelling of the ground substance, changes in the spatial arrangement of the fibrils occur with resultant clouding. This theory gives us a framework in which the mucoid and the regularity of the corneal structure can be related to its transparency (Smelser, 1958).

Cogan adds a thought that perhaps some of the transparency has been ascribed to the presence of the polysaccharide that is present abundantly in the cornea.

Physiology

Turgescence and transparency

Cogan states that there is no tissue of the body, with the possible exception of Wharton's jelly in the fetus, which when removed and placed in an aqueous solution has the capacity to swell as does corneal tissue. The magnitude of this swelling amounts to several hundred per cent its normal state and is effectively independent of any variations in tonicity or hydrogen-ion concentration that might be expected to occur physiologically. Moreover, it swells in those fluids with which it can reasonably be expected to be in contact normally, that is, aqueous humor and blood plasma, quite as well as it does in solutions of electrolytes. By contrast, the fresh sclera shows practically no swelling when placed in similar solutions.

The reason for this excessive water-binding capacity of the cornea in comparison with that of the sclera is apparently the specific polysaccharide in the cornea, for when this is removed by prior treatment with alkali, the cornea shows little capacity to take up water over and above the amount which it contains normally.

Chemistry of cornea

The cornea and sclera from a chemical constitution standpoint are very similar. Adler states:

Protein

The most striking component of each is the protein content which consists largely of five different proteins: namely, mucoid, collagen, elastin, albumin, and globulin. Collagen, which breaks down into gelatin on boiling, is present in greatest concentration in the cornea. It is one of the factors involved in wound healing, and a delay in its formation has been demonstrated in vitamin-C deficiency. Mucoid, which is rich in carbohydrate, was isolated from the cornea many years ago by Moerner and later by Karlberg. These authors believed that mucoid contained no sulfate radical. It remained for Meyer and Chaffee to determine the chemical nature of mucoid. They concluded that it is a natural monosulfuric acid ester of hyaluronic acid. The failure of previous authors to identify it as a sulfuric acid ester was due to their method of extraction. It is not identical with heparin, since this substance is hydrolyzed by an enzyme called hyaluronidase, whereas heparin cannot be hydrolyzed.

The structure of the collagen which consists principally of long polypeptide chains, from different tissues, has been studied by means of roentgen ray diffraction patterns, which have brought out certain differences between collagen and gelatin. Also in the cornea there are mucoprotein, elastin, albumin and globulin found, and amino acids have been isolated by Jess, histidine, arginine, and lysine.

There is a striking difference in the nucleoprotein content of the epithelium and the stroma. Nucleoprotein is found in high concentration in the epithelium due to the higher concentration of cells in the epithelium, compared to that in the stroma.

Lipids

One of the major chemical differences between the corneal stroma and the epithelium is their lipid content, which is about a hundred times greater in the epithelium than in the stroma. The cholesterol content increases with age and accounts for the incidence of arcus senilis in old people. The lipid content of the epithelium probably accounts for the preferential permeability of the epithelium to fat soluble substances.

Other organic substances

Both glutathione and ascorbic acid are present in the normal cornea. Henkes found 16 mg. percent glutathione and 24.1 mg. percent ascorbic acid in the cornea. The greatest concentration of ascorbic acid is in the epithelium. The concentration in the stroma is about that of the aqueous humor. Ascorbic acid is present as is riboflavin.

Inorganic constituents

Iron, copper, zinc and manganese. Christensen states:

"The bulk of the cornea is composed of connective tissue which consists of two principal factors, a protein collagen and ground substance. The stromal lamellae are collagen and, in turn, are bound by ground substance. The ground substance is a gel supported by fibrillar or lamellar fabric consisting of proteins, mucopolysaccharides, and probably mucoids or glycoproteins. At the present time most investigations have been concerned with the mucopolysaccharides, which contain at least three known fractions, chondroitin, chondroitin sulphate, and keratosulphate. This latter term was coined by Meyer to designate the mucopolysaccharide peculiar to the cornea. Chondroitin has many properties similar to hyaluronic acid. The mucopolysaccharide concentration in the cornea differs from that of the sclera as well as from the connective tissue of most other systems. In studies of radioactive sulphur uptake and elimination between corneal stroma and that of the sclera, the corneal uptake and elimination is slower. One explanation attributes this to the increased concentration of keratosulphate in the cornea. At least four soluble corneal protein fractions have been found by electrophoretic studies in the corneal epithelium. Descemet's membrane is a collagen tissue that differs from corneal stroma in morphology, swelling characteristics, solubility in acid, and in the type of polysaccharides."

Carbohydrate and protein complexes

Combinations of protein and carbohydrate form complexes, divided into glycoproteins and mucopolysaccharides. The glycoprotein are found in (1) blood group antigens, (2) complement and (3) the gonadotrophic hormone of pregnancy. The mucopolysaccharides contain hexosamines loosely joined to protein. The two most important mucopolysaccharides in the body are hyaluronic acid and chondroitin-sulfuric acid. The ground substance of connective tissue is rich in mucopolysaccharides. The complex is also found in the mucoid material of the umbilical cord (Wharton's jelly), the matrix of cartilage and the vitreous humor of the eye. Heparin is also a mucopolysaccharide, as is the cement substance between cells. Mucopolysaccharides can be hydrolized by enzymes such as the hyaluronidases with a resultant loss of tissue integrity leading to the potentiality of spread of infection and tumor. Mucopolysaccharides are also found in the mucinous and mucoid secretions of epithelial and mesenchymal cells. The epi-

thelial mucopolysaccharides, called mucin, may have pathologic processes which affect these tissues and may be accompanied by the elaboration of excessive amounts of mucin (Robbins, 1957).

It is interesting to know that the anterior pituitary is consistently involved in the condition of gargoylism (or Hurler's disease) which, on chemical investigation, is now interpreted as a generalized metabolic disturbance in which the cells of many tissues and organs retain large amounts of mucopolysaccharide (Anderson, 1957).

Metabolism of cornea

Inasmuch as the eye is closed during the night and open during the day oxygen which might be obtained during exposure is certainly excluded at night and must therefore be obtained through the limbal capillaries and from the, let us say, aqueous humor. Adler states: "It must also be able to derive its metabolites by diffusional processes from the limbal capillaries, the aqueous humor and the tears." The relative contribution of these three sources is not known. Robbie, Leinfelder, and Duane have also pointed out that most of the O_2 consumption by the cornea is due to the epithelium.

Corneal permeability and nutrition

Cogan states, "The corneal stroma (including Descemet's membrane) is freely permeable to aqueous solutes and shows no significant resistance to the passage of an electric current over that of an equal thickness of physiologic sodium chloride solution."

Permeability of the corneal epithelium is influenced by the action of nerves as is evidenced by the edema present in the cornea in neurotrophic disturbances, according to Thomas. Loewenstein has shown that certain chemicals will go into the eye but will not come out. After irritation of the trigeminal nerve, the amount of water in the epithelial cells is increased and after section of the trigeminal nerve, the epithelium becomes edematous and islands of cells were desquamated leaving pits in the corneal surface.

Cogan found that the various substances tested could be classified as: "(1) those which pass through the stroma much more easily than through the epithelium and stroma; (2) those which pass through the stroma and the epithelium-stroma about equally, and (3) those which do not pass through either. It was observed that some substances capable of penetrating the epithelium-stroma combination are of larger molecular size than many nonpenetrating substances. Conversely, the permeability of the isolated stroma apparently is dependent on molecular size. The epithelium retains electrolytes and also many nonelectrolytes, and there is no exchange of ions when different salts are present on each side of the cornea. The permeability of the cornea depends also on differential solubilities. Substances which characteristically pass through the stroma but not through the epithelium are usually insoluble in fats. Those which pass equally through the stroma and the epithelium and stroma are generally soluble in both water and fat. Those which do not pass through either layer have large molecules (e.g., proteins) or are insoluble in water."

In this connection the cornea receives its nourishment by a process of simple diffusion of the intraocular fluid from the periphery. It will be remembered that this fluid dialyses from all the blood vessels in the eye; and the evidence points to the fact that diffusion may take place directly from the vascular plexus around the limbus or indirectly from the fluid in the anterior chamber. The metabolism of the cornea is extremely slow and it appears that sufficient nourishment can be obtained if one or other of these two routes is utilized (Duke-Elder).

Healing processes

Smelser states:

> Ground substance is present in all connective tissues. It is related anatomically to the fibers, but by an unknown means. During the past 20 years

the chemistry of connective tissue ground substance has made great strides, primarily through the research of Karl Meyer and his associates, who have demonstrated the presence of three acid mucopolysaccharides (keratosulfate, chondroitin sulfate A, and chondroitin), as major constituents in the cornea. Because the chemistry of these compounds has been well studied, their metabolism is beginning to be understood. They are far from inert, but are rather in a state of constant "turn-over." For example, 50 percent of the hyaluronic acid in rabbit skin is destroyed and re-formed every 3.7 days. The sulfated mucopolysaccharides of skin have a longer half-life—50 percent being "turned-over" every 7.7 days.

The cornea contains an apparently unique keratosulfate in addition to chondroitin sulfate A and chondroitin, and the "turn-over" time of the sulfated corneal mucopolysaccharides is much longer than the equivalent periods for other tissues. Apparently, 50 percent of the sulfated materials in the cornea are replaced once in approximately 32 days. This figure may be exaggerated due to the possibility that freed sulfate radicals may not diffuse out of the cornea before they are re-synthesized into a new molecule of some sulfated mucopolysaccharides, thus introducing an error in the calculation of the turn-over rate. The marked difference in the turnover rate of sulfated mucopolysaccharides of the cornea and other connective tissues has been demonstrated.

Radioautographs were prepared of the cornea, sclera and an artery of rabbits which had received isotopically labelled sodium sulfate 1, 6, 12 and 24 days prior to autopsy. All three tissues incorporated considerable amounts of radioactive sulfate, but in cases of sclera and artery the sulfated material was rapidly replaced so that by 24 days almost all of the sulfate contained in them had been supplanted by new non-radioactive material. The cornea, however, retained a large proportion of the radioactive sulfate even after 24 days.

The slow turn-over rate of a fundamental corneal constituent allows an old problem to be attacked: the question of replacement of corneal grafts. We still do not know if a corneal graft retains its donor identity or is slowly replaced by the host. This question which was so real when propounded originally, has lost much of its significance, for today we do not regard any tissue as absolutely static and unchanging. The normal cornea is always being renewed, although at a slow rate. Dohlman has labelled donor corneas with radioactive sulfate which was incorporated as a part of the corneal ground substance. Such corneas were used as transplant donors. He was able to show that except for an initial partial replacement of the sulfated compounds in the graft, the bulk of the original sulfated mucopolysaccharide of the transplant remained, indiciating that this component of the original donor tissue persists in the transplant.

These constituents of the ground substance are relatively easily removed from the fibers without recourse to drastic chemical treatment, since the forces linking collagen fibers and acid mucopolysaccharides are weak. Mucopolysaccharides are clearly related to fiber formation, both in embyronic development of the cornea and in the healing of its wounds, but the significance of this relationship has not yet been elucidated. They increase in amount when fibers form, and have been implicated as a necessary factor in the production of collagen fibers *in vitro*, but there is, as yet, no conclusive evidence that the presence of these materials is necessary for *fibrillogenesis in vivo*.

The quantity and location of the sulfated mucopolysaccharides, keratosulfate, and chondroitin sulfate, can be determined in tissues by radioautographic techniques. Sulfated materials appear very early in a healing wound, and their rate of synthesis is and remains high for a long time in the areas of the regenerating connective tissue. While the healing process involves the formation of sulfated mucopolysaccharides, the preceding injury, i.e., the surgical incision, causes some reduction in the ground substance in the adjacent tissue. For this reason, at the initiation of the healing process there is probably a deficit in ground substance materials in the cornea surrounding the wound. This can be seen by examining sections of an early corneal wound stained with toluidine blue. The ground substance constituents stain metachromatically with this dye and one can see that metachromasia is reduced in the tissue near a cut. Of course, this area is also subject to swelling which of itself would weaken the intensity of the staining reaction. However, the change appears to be of a magnitude too great to be explained by swelling alone, thus indicating a reduction in the absolute amount of ground substance.

Fibroblasts

These probably arise from cells already present in connective tissue, that is, from connective tissue cells. These cells enlarge and migrate from surviving tissue into the defect, especially if blood is present, where they have been preceded by polymorphonuclears and monocytes. As fibroblasts move into the area they appear bipolar, but later they put out processes in various directions, and these processes fuse with those of neighboring fibroblasts. Mucopolysaccharides are laid between the cells and combine with a protein secretion of the fibroblasts to form collagen fibers. These lie extracellularly in parallel rows, between which are found the now shrunken cells; they become mature connective tissue cells, and lie dormant until stimulated to renewed activity. The fibers laid down in this way form sheets of collagen,

and several such sheets may be formed one on another. The direction in which the fibers of each sheet are oriented is determined by the tensions to which they are subjected. After fixation and staining, fibroblasts, as compared with monocytes, have a less sharp and clear outline. In the nucleus, the chromatin is concentrated along the nuclear membrane and in one or two nucleoli. Whereas the macrophage in inflammatory tissue commonly contains phagocytized cells and debris, the fibroblast is generally not phagocytic (Anderson, 1957).

Endothelium

While fibroblasts are migrating, changes appear in the capillaries at the edge of the wound if this is near the limbus. Here the endothelial cells swell and divide, thus forming a bud that projects from the capillary and then begins to migrate. The path of migration is not, however, a straight line, such as is followed by fibroblasts, but rather an arc of a circle. Following this curving path, the endothelial cells meet other similar cells also traveling in an arc (now this is not the endothelium of the cornea but endothelial cells in and around blood vessels at the limbus), and the two fuse, forming a loop that is attached to capillaries at both ends. Granulation tissue eventually forms consisting of (1) newly formed capillary loops, (2) fibroblasts, (3) leukocytes. And, as you know, this tissue bleeds freely, is insensitive, lacking nerves, and is resistant to infection, because it contains many mononuclear phagocytes (Anderson, 1957).

Epithelium

The cells of the epithelial type are firmly attached to each other and on the epithelium of the skin they migrate as sheets of cells in contrast to fibroblasts which migrate separately. The mechanism of the locomotion of this epithelial sheet appears to depend on ameboid motion of its constituent cells. In order to migrate, epithelium requires a substratum of mesenchyme and if the mesenchyme remains intact the epithelium may begin to migrate at once. Thus in wounds of the cornea limited to the epithelial layer, these cells move in from the sides and may close the defect in as short a time as six hours. Closure here is accomplished solely by cell migration; cell division plays no part. The same is true of closure of small wounds of the skin (Anderson, 1957).

Cell division

Cell division aids migration in replacing epithelium lost in extensive wounds. Of course, even in normal epithelium, mitosis occurs, as old cells must continually be replaced by new ones. After a wound, mitosis appears at first to be depressed and in the initial stage of migration there may be no cell division. Later, especially in larger wounds, mitoses become abundant. In experimental wounds they have been observed to be twice as numerous as in resting skin. Also, cell division may continue after migration has ceased with the closure of the wound, until all the cells have been replaced.

Replacement of lost cells and tissues

Replacement of lost cells and tissues is accomplished by two processes, cell migration and cell proliferation. In wounds of the skin, the cells chiefly concerned are fibroblasts, vascular endothelium, and epithelium. These types of cell have the property, on appropriate stimulation, of swelling and reassuming their embryonal appearance; they become actively motile and undergo mitotic division (Anderson, 1957).

Factors influencing healing

Extensive studies have been made on the influence of various drugs on corneal healing. Quoting from a personal communication from Irving Leopold in Philadelphia:

"There are certain drugs which may have some influence on wound healing. Many agents will interfere with wound healing. These include antibiotics, local anesthetics and steroids. There are many agents which

probably interfere but haven't been tested. Almost any ointment vehicle will interfere with corneal regeneration and, to some extent, with stromal proliferation. I know of no agents which, in our hands, have been known to speed up on healing although there are references in the literature on the use of Chlorophyll for this purpose as well as certain of the amino acids."

During the past six or eight years some change has taken place in the effect of Cortisone on wound healing. Originally it was condemned vigorously, but more recently except in types of herpetic lesions Cortisone or steroids have been given a better bill of health from a retardation factor in corneal healing.

All types of local anesthetics have a retarding effect on corneal healing and must be vigorously condemned. This condemnation as portrayed by some of the slides which are presented carries over into the field of corneal healing not only from a surgical standpoint, traumatic injuries to the cornea, but infectious processes of the cornea by various disease processes. Adler has pointed out that some of this may be caused by their detergent action. Some of the anesthetics commonly used are good wetting agents. Strong detergents cause an inhibition of epithelization and if the anesthetic drugs are incorporated in ointments in which their detergent action is reduced they fail to delay some migration at the same extent as in aqueous solutions.

In studying the effect of various local antiseptics on the regeneration of corneal epithelium in rabbits, Bellows used mild silver protein (10 percent); zinc sulfate (0.5 percent); Zephiran chloride (1:3000); acriflavine (1:000); metaphen (1:2500); merthiolate (1:2500); oxycyanide of mercury (1:5000); and various sulfonamides and penicillin. All these drugs had a considerable delaying effect on the healing of the corneal epithelium, in rabbits. Bellows concluded that the aqueous solutions of sodium sulfathiazole or penicillin caused the least traumatizing effect (Thomas, 1955). One of the greatest inhibitors is cocaine.

Friedenwald, Buschke and Moses made a study concerning the vitamin-A deficiency on the healing of corneal wounds, in their experiments these authors attempted to differentiate between mitotic activity, nonmitotic cell movement and the beneficial effects of vitamin A on secondary infection, in their efforts to explain the differences of opinion regarding the effects of vitamin A that had previously been expressed by some authors. In experiments on rats it is found that there is a profound, though variable, inhibition of mitotic activity in the corneal epithelium in the presence of vitamin-A deficiency.

There is some discrepancy with reference to the findings concerning the various antibiotics streptomycin, chloramphenicol, aureomycin, terramycin, achromycin, penicillin, and so forth, on healing. It is our opinion that all of these decrease and deter the healing of the cornea. It has been found that anesthetics incorporated in an ointment cause less delay than when they are in an aqueous solution, but the reverse is true in the case of antibiotics; for the antibiotic in an aqueous solution does not seem to inhibit the healing process as much as when incorporated in an ointment.

Herrmann and his associates have found that there are certain substances which cause loosening of the epithelium; these included detergents, such as butyl alcohol, enzymes, certain metabolic poisons, local anesthetics, freezing and mustard gas. Loosening of the corneal epithelium was also caused by histamine. Inorganic salts and a wide variation in pH had little effect on the adhesion of the corneal layers. The effect of detergent substances suggests that the cohesive boundary includes a lipid component, and the action of enzymes indicates a protein component also.

Factors stimulating corneal healing

Low dosages of radiation from an unfiltered quartz mercury arc stimulated mitotic

activity, but larger doses seemed to inhibit it, according to the work of Buschke, Friedenwald and Moses.

Schaeffer studied the effect of certain amino acids on the healing of experimental corneal wounds using a mixture containing cystine, proline, asparagin and glutamine; the pH of the preparation was 7.2. Complete regeneration of the corneal defect in the eyes treated with the amino acids occurred within 12 to 42 hours; in control eyes, healing was not complete for 55 to 120 hours. deBerardinis and Bonavolonta (1951) tested various individual amino acids as to their effect on the healing of corneal wounds. They found that arginine, histidine and cysteine applied locally increased the rate of repair of superficial and deep corneal wounds in the rabbit. Asparagin, phenylalanine, glutamine, tryptophan and proline were less effective in promoting healing. These authors observed that the respiratory rate of the cornea increased in the presence of cysteine, histidine, arginine, asparagin and phenylalamine. Hence they concluded that the healing action of amino acids very probably depends on their effect on the oxidative processes.

D'Ermo studied the effect of para-aminobenzoic acid (vitamin H) on experimental wounds of the cornea in rabbits. He showed that there was considerable increase of proliferative activity in the corneal parenchyma after six or seven days of treatment. From the second to the fourth day, the proliferative activity of the epithelium and of the parenchyma was nearly the same as that of the controls.

It has also been shown that the repair of collagenous tissues is dependent on an adequate supply of ascorbic acid. Deep corneal ulcers in patients were also shown to heal more rapidly under similar conditions with ascorbic acid, but no difference was observed in the healing of superficial lesions of the cornea in patients treated with vitamin C.

We concur with the report of Newell concerning the stimulation of corneal epithelization in experimental animals by topical application of erythrocytes. We believe that they contain a large amount of adrenosine triphosphate which may furnish the high-energy phosphate bonds necessary for increased corneal metabolism which accompanies accelerated healing. The potassium ion in the red cell could catalyze such a reaction and perhaps other catalysts also are present to aid in the stimulation of corneal metabolism. We have personally used laking of blood in the eye to stimulate corneal epithelization.

At the present time we have under investigation the following:

1. Chymar (chymotrypsin) to be used both systemically and topically; the latter will be done first on animal experimentation and then subsequently on human eyes if there is no untoward reaction.

2. Streptokinase-streptodornase (Varidase) which is a purified and concentrated combination of enzymes and which acts indirectly by activating the normal fibrinolytic enzyme in human serum that acts upon a substrate of fibrin or fibrinogen. This will be carried on in conjunction with the chymotrypsin investigation. We anticipate using this subconjunctivally to ascertain its effect upon corneal healing.

3. Continued laking of whole blood in the conjunctival sac and covering the cornea; the effect of injecting whole blood subconjunctivally. In conjunction with this plasma also should merit consideration.

4. Lid suturing, which has been utilized in corneas which have failed to epithelize following chemical and heat burns of various types. The question arises regarding the lack of oxygen in such cases, and if the lids are sutured in one involved eye should both eyes be bandaged for a period of days or weeks? So far we have not tried this in animal experimentation.

5. Oxygen, which should be discussed with any type of corneal healing. Here again we are confronted with a problem regarding bandaging of eyes in the process of both

intentional surgical procedures (keratoplasty, cataract surgery, and so forth) and accidental trauma, infections and burns. Perhaps we have decelerated corneal healing in these cases by too much bandaging. It is quite possible that we should attempt to accelerate healing by more oxygenation.

6. Too little investigation, so far as we are able to ascertain, has been done on the various types of estrogens and gonadotrophic preparations. What effect these would have given both systemically and topically is an unsolved problem; it merits definite critical investigation, as do some of the other phases of investigation mentioned herein.

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THERAPEUTIC STUDIES IN EXPERIMENTAL CHEMICAL INJURY OF THE CORNEA*

III. Corticosteroid studies

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The second stage in the genesis of corneal chemical injury is that of repair, or the removal of necrotic tissue and toxic products, and their replacement by viable cells. This is accomplished by inflammatory cell infiltration, neovascularization, and finally cicatrization. Unfortunately, these processes so alter the structure of the cornea as to circumvent its primary function of regularly transmitting and refracting light. Healing, in the usual sense, with scarring therefore is not entirely beneficial, from the standpoint of visual function. Ideally, a corneal injury should be induced to heal without derangement of the corneal lamellas, without cicatrization, and without vascularization. Unless this is accomplished, corneal opacification results, and visual function is impaired.

Theoretically, removal of the influence of necrotic tissue upon phagocytic elements, that is, the elimination of inflammatory cell response, should reduce the final amount of scarring, because this occurs following lamellar separation by fluid and inflammatory cells. The anti-inflammatory properties of the corticosteroid drugs have been widely recognized, and employed in intraocular inflammatory processes as well as elsewhere in the body. Since surface burns of the eye are so readily amenable to topical medication, and since corticosteroids are readily available for topical ophthalmic use, this series of experiments was designed to evaluate the anti-inflammatory properties of cortisone, hydrocortisone, and prednisolone, when applied to chemically induced lesions of the rat cornea.

The experimental procedure followed was essentially the same as previously reported in the Calsulfhydryl and Neutralize studies. Albino rats of the Wistar strain, weighing about 100 grams were chosen as the experimental animal because previous studies have indicated that their corneas are easily subject to slitlamp examination, and because they respond in very much the same way to injury as do other larger, less readily available experimental animals. Burns with representative mineral acids and alkalis were produced with the splash technique, as previously described. The course followed by the burned corneas was evaluated biomicroscopically at regular intervals, until the healing processes had become static. Statistical evaluation of the data, as recorded according to our modification of the Friedenwald scheme, included comparison of average "scores" for treated and untreated eyes, application of the Fisher "t" test, and determination of significance probability.

The experimental results were as follows:

Experiment I

Both eyes of 15 albino rats were burned with normal sodium hydroxide, one drop in each eye every three seconds for one minute. Both eyes were then irrigated with distilled water for one minute and 0.05 cc. Cortef acetate (1.5 percent) was injected subconjunctivally into the right eyes. The left eyes were followed as controls. Both eyes were then examined at 24-hour intervals with the slitlamp. The results are summarized in Table 1.

Conclusions. Subconjunctival injection of Cortef acetate did not significantly alter the

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TABLE 1

COMPARISON OF "CORTEF ACETATE" (SUBCONJUNCTIVALLY) TREATED RIGHT EYE WITH UNTREATED LEFT EYE FOLLOWING STANDARD BURN OF EACH EYE WITH 1N NaOH

Hours after Injury	Average Score Treated Eye	Average Score Control Eye	Average Difference	t	P
24	23.800	23.266	−0.534	0.888	0.40
48	24.200	23.000	−1.200	3.054	0.01
72	22.666	22.800	+0.134	0.316	0.80
96	21.866	22.066	+0.200	0.566	0.60
120	24.467	27.867	+3.400	4.082	0.001
168	28.333	28.466	+0.133	0.263	0.80
216	27.800	28.133	+0.333	0.750	0.50

course of 1N sodium hydroxide induced corneal burns, as compared with similarly burned, untreated eyes. The degree of scarring and vascularization was essentially the same in the treated and control eyes.

EXPERIMENT II

Both eyes of 15 albino rats were burned with 2N nitric acid as in the first experiment, for one minute. Both eyes were then irrigated with distilled water for one minute and 0.05 cc. of 1.5-percent Cortef acetate suspension was injected subconjunctivally into the right eyes. The left eyes served as controls. The results of this experiment are shown in Table 2.

Conclusions. Subconjunctival injection of Cortef acetate suspension did not significantly alter the course of 2N nitric acid burns of the rat corneas. Final scarring and vascularization were present to the same extent in the treated and untreated eyes.

EXPERIMENT III

Both eyes of 15 albino rats were burned with 2N nitric acid for one minute, and were then irrigated with distilled water for one minute. One drop of Cortef acetate suspension was instilled into the right eyes of the animals three times daily until no further healing changes could be noted. The left eyes were followed as controls. The treated data is presented in Table 3.

Conclusions. Topical instillation of 1.5-percent Cortef acetate suspension resulted in slightly more rapid healing of nitric acid induced corneal burns, although statistically, the difference between treated and untreated eyes was not significant.

EXPERIMENT IV

One drop of 1N sodium hydroxide solution was instilled into each eye of 15 albino rats every three seconds for one minute, thereby producing a standard burn. Both eyes were then irrigated for two minutes with distilled water. Cortef acetate suspension was instilled into the right eyes three times daily until no further change could be noted. Table 4 depicts the results of this experiment.

Conclusions. Topical instillation of Cortef

TABLE 2

COMPARISON OF "CORTEF ACETATE" TREATED RIGHT EYE WITH UNTREATED LEFT EYE FOLLOWING STANDARD BURN OF EACH EYE WITH 2N HNO_3

Hours after Injury	Average Score Treated Eye	Average Score Control Eye	Average Difference	t	P
24	19.800	20.333	+0.533	1.166	0.30
48	22.200	22.333	+0.133	0.306	0.80
72	21.113	21.200	+0.087	0.207	0.80
96	20.466	20.733	+0.267	0.727	0.50
120	24.467	24.467	0.000	0.000	1.00
168	26.733	25.200	−1.533	1.500	0.20
216	26.133	25.667	−0.466	0.439	0.70

TABLE 3

COMPARISON OF "CORTEF ACETATE" (TOPICAL) TREATED RIGHT EYE WITH UNTREATED LEFT EYE FOLLOWING STANDARD BURN OF EACH EYE WITH 2N HNO_3

Hours after Injury	Average Score Treated Eye	Average Score Control Eye	Average Difference	t	P
24	16.400	18.000	+1.600	2.903	0.02
48	16.667	19.600	+2.933	6.240	0.001
72	16.467	19.667	+3.200	3.828	0.01
96	15.467	18.533	+3.762	3.762	0.01
144	12.333	14.867	+2.534	1.721	0.10
192	11.467	15.000	+3.533	2.003	0.05
240	11.733	15.600	+3.867	2.388	0.05

TABLE 4

COMPARISON OF "CORTEF ACETATE" (TOPICAL) TREATED RIGHT EYE WITH UNTREATED LEFT EYE FOLLOWING STANDARD BURN OF EACH EYE WITH 1N NaOH

Hours after Injury	Average Score Treated Eye	Average Score Control Eye	Average Difference	t	P
24	20.066	21.200	+1.134	1.877	0.10
48	20.866	22.266	+1.400	2.997	0.01
72	21.400	22.600	+1.200	2.992	0.01
96	20.733	21.933	+1.200	3.045	0.01
144	21.333	23.533	+2.200	5.188	0.001
192	24.600	26.066	+1.490	3.490	0.01
240	23.866	25.133	+1.267	2.953	0.02

TABLE 5

COMPARISON OF "NEO-CORTEF" TREATED RIGHT EYE WITH UNTREATED LEFT EYE FOLLOWING STANDARD BURN OF EACH EYE WITH 2N HNO_3

Hours after Injury	Average Score Treated Eye	Average Score Control Eye	Average Difference	t	P
24	11.866	13.266	+1.400	3.608	0.01
48	12.400	15.266	+2.866	3.647	0.01
72	13.133	16.533	+3.400	7.439	0.001
96	12.000	16.200	+4.200	3.414	0.01
144	10.133	15.866	+5.733	4.399	0.001
168	8.333	14.400	+6.067	4.822	0.001
192	5.933	13.000	+7.067	4.810	0.001
216	6.066	12.200	+6.134	4.210	0.001

acetate in the treatment of sodium hydroxide burns of the rat cornea failed to significantly increase healing rate, or reduce final scarring and vascularization as compared with similarly burned, untreated eyes.

EXPERIMENT V

Standard corneal lesions were produced in both eyes of 15 albino rats with 2N nitric acid according to the technique described above. One drop of NeoCortef (hydrocortisone) was instilled into the right eyes of the animals three times a day until no further change was observed. The left eyes were followed as controls, and the animals were examined at 24-hour intervals. Statistical analysis of the data is presented in Table 5.

Conclusions. Animal eyes treated with NeoCortef healed significantly faster, and with less residual scarring and vascularization than did similarly burned untreated eyes. NeoCortef suspension was found to be of definite value in the treatment of nitric acid burns of the rat cornea.

TABLE 6

COMPARISON OF "NEO-CORTEF" TREATED RIGHT EYE WITH UNTREATED LEFT EYE FOLLOWING STANDARD BURN OF EACH EYE WITH 1N NaOH

Hours after Injury	Average Score Treated Eye	Average Score Control Eye	Average Difference	t	P
24	25.000	24.800	−0.200	0.514	0.60
48	23.866	24.400	+0.534	1.312	0.20
72	22.666	22.466	−0.200	0.386	0.70
96	19.733	21.400	+1.667	3.157	0.01
144	22.000	24.000	+2.000	2.691	0.02
168	24.000	25.200	+1.200	1.000	0.40
192	24.133	25.200	+1.067	1.403	0.20
216	24.133	24.466	+0.333	0.353	0.80

EXPERIMENT VI

Standard corneal lesions were produced in 15 rats with 1N sodium hydroxide solution. Both eyes were irrigated with distilled water for two minutes. One drop of NeoCortef was instilled into the right eyes three times a day until healing was complete. The results are presented in Table 6.

Conclusions. No significant difference was noted between the healing rate of sodium hydroxide burned eyes when treated with NeoCortef and untreated control eyes.

EXPERIMENT VII

Standard corneal lesions were produced in both eyes of 15 rats with 1N sodium hydroxide solution. Both eyes were irrigated with distilled water. One drop of NeoCortef suspension in castor oil (2.0 μg. per drop) was instilled into the right eyes once daily. The left eyes were followed as controls. The results are depicted in Table 7.

Conclusions. Suspension of NeoCortef in castor oil failed to enhance the drug's anti-inflammatory action in this experiment. Sodium hydroxide burns of the cornea healed at the same rate, and with the same degree of scarring when treated with NeoCortef-caster oil suspension as did untreated eyes.

EXPERIMENT VIII

Standard corneal lesions were produced in 15 rat corneas with 2N nitric acid. Both eyes were then irrigated with distilled water for one minute. NeoCortef suspended in castor oil was instilled into the right eyes once daily until healing was complete. Statistical data is presented in Table 8.

Conclusions. Rat eyes burned with 2 N-nitric acid healed significantly faster and with less residual scarring and residual scarring and vascularization than did similarly burned, untreated eyes, when treated with NeoCortef-castor oil suspension. No significant difference, however, was noted between nitric acid burned eyes treated with aqueous

TABLE 7

COMPARISON OF RIGHT EYE TREATED WITH "NEO-CORTEF" IN CASTOR OIL WITH UNTREATED LEFT EYE FOLLOWING STANDARD BURN OF EACH EYE WITH 1N NaOH

Hours after Injury	Average Score Treated Eye	Average Score Control Eye	Average Difference	t	P
24	18.933	20.667	+1.734	4.751	0.001
48	18.867	20.333	+1.466	5.090	0.001
72	18.533	18.867	+0.334	1.392	0.20
96	16.600	17.133	+0.533	1.444	0.20
144	14.333	17.467	+3.134	2.954	0.02
192	15.933	19.267	+3.334	2.576	0.05
240	17.400	19.800	+2.400	1.878	0.10

TABLE 8

COMPARISON OF RIGHT EYE TREATED WITH "NEO-CORTEF" IN CASTOR OIL WITH UNTREATED LEFT EYE FOLLOWING STANDARD BURN OF EACH EYE WITH 2N HNO_3

Hours after Injury	Average Score Treated Eye	Average Score Control Eye	Average Difference	t	P
24	16.133	16.866	+ 0.733	3.425	0.01
48	14.667	17.800	+ 3.133	5.420	0.001
72	14.000	18.333	+ 4.133	6.966	0.001
96	11.267	14.800	+ 3.533	3.743	0.01
144	6.933	14.600	+ 7.667	6.470	0.001
168	4.733	14.867	+10.134	7.813	0.001
192	3.000	12.467	+ 9.467	4.900	0.001
216	1.333	11.000	+ 9.667	5.422	0.001
288	0.933	11.000	+10.067	5.899	0.001

NeoCortef suspension and the castor oil suspension.

EXPERIMENT IX

Standard burns were produced in both eyes of 15 rats with distilled water and 0.1 cc. of Solu-Cortef, containing 5.0 mg. per cc. of hydrocortisone was injected subconjunctivally into the right eye of each animal. The left eye of each animal served as an untreated control. Results of this experiment are presented in Table 9.

Conclusions. Corneal burns in rat eyes produced with 2N nitric acid, and treated with subconjunctival hydrocortisone, healed at the same rate as untreated eyes, and with the same amount of scarring.

EXPERIMENT X

Both eyes of 15 albino rats were burned with 1N sodium hydroxide solution for one minute. Both eyes were irrigated with distilled for one minute and 0.1 cc. of a solution of Solu-Cortef (5.0 mg. cc.) was injected subconjunctivally into the right eye of each animal. The left eyes were followed as untreated controls. The results of this experiment are shown in Table 10.

Conclusions. Subconjunctival injection of Solu-Cortef failed to decrease the rate of healing of rat corneas burned with sodium hydroxide, nor did it reduce the amount of scarring and vascularization. No significant difference was noted between the treated and untreated eyes.

EXPERIMENT XI

Both eyes of 15 albino rats were burned with 1N sodium hydroxide for one minute. Both eyes were then irrigated with distilled water for one minute. One drop of Hydeltrasol (prednisolone 21 phosphate) 0.5-percent solution was instilled into the right eyes three times a day. The left eyes were followed as untreated controls. Statistical anal-

TABLE 9

COMPARISON OF "SOLU-CORTEF" TREATED RIGHT EYE WITH UNTREATED LEFT EYE FOLLOWING STANDARD BURN OF EACH EYE WITH 2N HNO_3

Hours after Injury	Average Score Treated Eye	Average Score Control Eye	Average Difference	t	P
24	16.533	16.467	−0.066	0.243	0.90
48	16.800	17.000	+0.200	0.468	0.70
72	16.133	17.200	+1.067	2.634	0.02
96	15.867	16.400	+0.533	1.156	0.30
144	10.867	11.133	+0.266	1.152	0.30
192	7.867	8.400	+0.533	2.776	0.02
240	2.600	3.600	+0.400	1.342	0.30

TABLE 10

COMPARISON OF "SOLU-CORTEF" TREATED RIGHT EYE WITH UNTREATED LEFT EYE FOLLOWING STANDARD BURN OF EACH EYE WITH 1N NaOH

Hours after Injury	Average Score Treated Eye	Average Score Control Eye	Average Difference	t	P
24	24.667	24.467	−0.200	0.258	0.80
48	24.133	23.800	−0.333	0.022	0.90
72	24.333	24.267	−0.066	0.372	0.70
96	25.000	25.267	+0.267	0.320	0.70
144	23.867	24.333	+0.466	1.183	0.30
192	23.000	23.533	+0.533	1.864	0.10
240	22.067	22.667	+0.600	1.749	0.20

TABLE 11

COMPARISON OF "HYDELTRASOL" TREATED RIGHT EYE WITH UNTREATED LEFT EYE FOLLOWING STANDARD BURN OF EACH EYE WITH 1N NaOH

Hours after Injury	Average Score Treated Eye	Average Score Control Eye	Average Difference	t	P
24	20.600	23.600	+3.000	5.122	0.001
48	21.800	23.600	+1.800	4.205	0.001
72	23.000	23.266	+0.266	1.330	0.30
96	24.466	24.833	+0.367	1.663	0.20
144	26.000	26.400	+0.400	2.220	0.05
192	25.071	25.500	+0.429	2.670	0.02
240	24.429	24.929	+0.500	3.110	0.01

TABLE 12

COMPARISON OF "HYDELTRASOL" TREATED RIGHT EYE WITH UNTREATED LEFT EYE FOLLOWING STANDARD BURN OF EACH EYE WITH 2N HNO_3

Hours after Injury	Average Score Treated Eye	Average Score Control Eye	Average Difference	t	P
24	18.200	19.400	+1.200	2.266	0.05
48	18.600	19.000	+0.400	1.079	0.30
72	16.833	17.633	+0.800	2.344	0.05
96	13.000	13.666	+0.666	1.456	0.20
144	9.800	9.666	−0.134	0.492	0.70
192	6.334	5.867	−0.467	1.605	0.20
240	2.067	1.933	−0.134	0.453	0.70

ysis of the results of this experiment are shown in Table 11.

Conclusions. Rat eyes burned with 1N-sodium hydroxide, and treated with prednisolone 21 phosphate healed at the same rate and with the same amount of scarring and vascularization as did similarly burned untreated eyes.

EXPERIMENT XII

Both eyes of 15 albino rats were burned with 2N nitric acid. Each eye was then irrigated with distilled water for one minute. Hydeltrasol drops were instilled into the right eyes three times a day until no further change was noted. The left eyes were followed as untreated controls. The results are shown in Table 12.

Conclusions. Rat eyes burned with nitric acid and treated with topical Hydeltrasol healed at the same rate and with about the same amount of final scarring and vascularization as did similarly burned, untreated eyes.

SUMMARY

A series of experiments was conducted to determine the effectiveness of certain topically and subconjunctivally administered corticosteroids in the management of representative acid and alkali burns of the rat cornea. Cortisone and prednisolone 21 phosphate failed to alter the course of sodium hydroxide and nitric acid burns of the rat cornea. Hydrocortisone was found to be significantly beneficial in nitric acid burns, when administered topically. Subconjunctival injections of hydrocortisone neither increased healing rate nor reduced the amount of final scarring and vascularization. Although the scope of this series of experiments is rather narrow, the results would tend to indicate that the anti-inflammatory action of corticosteroids at the tissue level is not marked in certain chemically induced corneal lesions.

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REFERENCE

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HETEROLOGOUS CORNEAL TRANSPLANTS IN RABBITS

A PRELIMINARY REPORT

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The work of Tucker[1] in culturing bovine tissues to reduce the antigenicity so that they might be used in human heterologous grafts and specifically his use of cultured bovine osseous and cartilaginous transplants in humans suggested the idea that culturing fetal bovine corneas in bovine plasma might reduce their antigenicity and produce less allergic reaction on transplantation.

Successful direct heterologous transplants in rabbits have been reported by Basu and Ormsby.[2] Kamata[3] reported a successful transplant of chick cornea into rabbit cornea after preserving the chick cornea in rabbit serum for three days prior to transplantation.

It was the purpose of this experiment to determine whether or not culturing fetal bovine eyes in bovine plasma reduces the allergic response of the host to the transplanted tissue.

METHODS AND MATERIALS

The donor material consisted of bovine fetal corneas obtained from the National Bone & Tissue Laboratories, Inc., Houston, Texas. Calves one to three months old weighing approximately 100 to 250 pounds were used. The eyes were removed under aseptic conditions in a sterile operating room upon opening the amniotic sac. The eyes were then either used directly in heterologous transplanting or stored in either 100 percent plasma or a 25 percent glycerine-75 percent plasma mixture at 40°F. To the plasma or glycerine-plasma mixture had been added penicillin and streptomycin.

The hosts were adult rabbits weighing 2.5 to 4.5 kg.

The rabbits were anesthetized with intraperitoneal nembutal. Pontocaine was dropped on the eyes and Xylocaine injected into the

TABLE 1

RESULTS IN GROUP 1

Rabbit Code No.	Epithelium	Endothelium	Stroma	Stroma
1A	3+	3+	0	1+
5B	3+	1+	1+	1+
6B	4+	4+	4+	4+
9B	3+	2+	2+	2+
10B	3+	2+	2+	2+
11B	4+	4+	4+	4+

superior and inferior fornices of both eyes. Superior and inferior rectus sutures were inserted after draping one eye. A small incision was then made in the center of the cornea perpendicular to a line joining the ear to the nose. Holding the edge of the incision with a forceps and using an iris spatula for dissection, interlamellar pockets were opened nasally and temporally extending to the limbus. Then fetal bovine corneal tissues, measuring 2.0 by 2.0 by 5.0 mm., were deposited in these pockets at the limbus. From one bovine cornea epithelium and stroma were placed nasally in the right eye, endothelium and stroma temporally in the right eye, and two pieces of stroma of the above dimensions nasally and temporally respectively in the left eye. Once in place, the bovine tissue did not migrate from the area in which it was deposited.

The grafts were observed daily for three days and Neosporin drops instilled daily during this period. The grafts were then observed every other day until maximum reaction occurred, then weekly till enucleation. In eyes showing an allergic response, observation was continued until clearing began to occur. In eyes showing no reaction to the grafts, observation was continued for at least two to three months.

Initially, suture was used to close the corneal incision; however, it was found that the suture itself caused reaction, vascular ingrowth to the suture, and predisposition to central corneal ulceration.

The reaction to the graft was graded from 0 (no reaction, graft clear) to 4+ (marked vascular ingrowth and dense leucoma at the site of tissue).

RESULTS

The types of transplants were divided into three groups. The results of each group are tabulated in Tables 1, 2, and 3.

GROUP 1

Six calf eyes not cultured (table 1). Fetal calf corneas tissue transplanted directly to rabbit corneas on the day obtained. The maximum reaction of all grafts of Group 1 was at 10-14 days.

GROUP 2

Seven calf eyes cultured at 40°F. in 100-percent bovine plasma and transplanted after

TABLE 2

RESULTS IN GROUP 2

Rabbit Code No.	Time Cultured	Epithelium	Endothelium	Stroma	Stroma	Time of Maximum Reaction
6A	3 da.	0	0	0	0	None
7A	3 da.	3+	3+	2+	2+	1 mo.
3A	1 wk.	0	0	0	0	None
8A	1 wk.	3+	1+	1+	0	1 mo.
4A	2 wk.	2+	0	0	0	2 wk.
5A	2 wk.	0	0	0	0	None
8B	1 mo.	3+	1+	1+	1+	1 mo.

TABLE 3

RESULTS IN GROUP 3

Rabbit Code No.	Time Cultured	Epithelium	Endothelium	Stroma	Stroma	Time of Maximum Reaction
3B	3 da.	0	0	0	0	None
4B	3 da.	2+	1+	1+	1+	2½ wk.
1B	1 wk.	3+	3+	3+	3+	2 wk.
7B	1 mo.	2+	0	0	0	2 wk.

culturing for various periods of time (two at three days, two at one week, and two at two weeks, and one at one month) (table 2).

GROUP 3

Five fetal calf eyes cultured in a 25 percent glycerine-75 percent plasma mixture at 40°F. (two transplanted at three days, two at one week, and one at one month) (table 3).

DISCUSSION

By the method described it was possible to compare the reaction of a rabbit to epithelium, endothelium and stroma separately. The results show, in all cases, that, when a reaction to the heterologous material occurs, the reaction is greater to epithelium than to either endothelium or stroma. A reaction to stroma or endothelium occurs frequently, but the degree of reaction is less than or equal to the reaction to epithelium in the same rabbit—never greater. Epithelium, therefore, incited an allergic response in the host more frequently and more markedly than tissue from the rest of the fetal bovine cornea.

Comparing the reactions to the cultured corneal tissue (Groups 2 and 3) with the allergic response to corneal tissue not preserved, the overall degree of reaction was less and the time of onset of the allergic reaction tended to be more delayed in tissues cultured in 100 percent bovine plasma or in a 25 percent glycerine-75 percent bovine plasma mixture. The usual time of onset of the allergic response of the rabbits bearing the uncultured calf corneas was 10 to 14 days. After culturing, the time of onset of the reaction might be delayed to as long as one month.

In three cases of culture in 100 percent plasma and in one case in 25 percent glycerine, there was no reaction to the grafts at all, the rabbit corneas remaining grossly clear even though the calf tissues were placed at the limbus.

An attempt was made to correlate the length of time the calf eyes were cultured with loss of antigenicity of the bovine cornea. However, no further significant decrease of antigenicity was found after three days of culture. Further studies on grafts from bovine eyes preserved over a longer period of time are in progress.

No significant difference was found between 100 percent plasma media and 25 percent glycerine-75 percent plasma media in effectiveness on reduction of antigenicity of the calf material. However, initial microscopic sections of donor calf corneas revealed better preservation of stroma tissue architecture in the 25 percent glycerine-75 percent plasma mixture than in the 100 percent plasma after three days of culturing. After one and two weeks or more of culturing in 100 percent plasma, the bovine stroma became edematous and its thicknes increased from a normal 1.0 mm. up to two to three mm. in thickness. This did not occur with the 25 percent glycerine mixture.

The hypothesis is set forth that preserving the calf eyes in bovine plasma or a mixture of glycerine and bovine plasma allows time for alteration of the protein constituents of the calf cornea and that these alterations are of such a nature that there

is a reduction of antigenicity of the tissues, allowing more successful heterologous transplants. Such an hypothesis might conceivably be applied to homologous as well as to heterologous grafts.

Summary

In heterologous transplants of fetal calf corneas into rabbit corneas, culturing the calf eyes in 100 percent plasma or a 25 percent glycerine-75 percent plasma mixture prior to transplanting the tissues reduces the intensity and degree of the allergic response of the host to the heterologous material.

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RECEPTOR AMBLYOPIA*

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Introduction

One may ask the question, what are the effects upon visual functions when the orientations of the retinal receptors, or their components are disturbed? Ordinarily we think of the receptors and their components as being in perfect alignment. However, it is not unusual when viewing a preparation to see the orientation of the receptors disturbed (fig. 1). While these disturbances are usually attributed to the manipulations associated with preparing the section, the author asks the reader to conceive that such disturbances may occur *in vivo*.

Disturbances in the orientation of aligned structures may also occur within the receptor cells themselves. An example† of such a phenomenon is shown in Figure 2, which is a photograph taken with an electron microscope showing the outer rod segments of an adult mouse. Magnification is ×11,400 and Dalton's fixative was employed. If one studies the transverse lamellar structure seen in these outer rod segments, one notes in one of the rods that this oriented structure is grossly disturbed. Disturbance of this magnitude is rare.

Further, it has been shown by Denton,[1,2] that the photopigment mo'ecules (visual purple) which lie in these transverse lamellar structures are also oriented. Of interest is the fact that with bleaching, the orientation properties of these molecules change to a certain degree.

* From the Department of Ophthalmology, Washington University School of Medicine. The experimental phases of the work discussed in this paper were completed while the author was associated with the Ohio State University, and that work was supported by a National Science Foundation Grant Number G 3877.

† I wish to express my appreciation to Dr. A. I. Cohen of the Department of Anatomy, Washington University School of Medicine, for allowing me to present this figure.

Thus, it is possible to conceive of a disturbance in the alignment of retinal receptors, or in the orientation of the internal structures, or photopigment molecules of the individual receptors. A method exists for studying the orientation factors in vivo in humans if visual fixation of the eye is assured. In 1933 Stiles and Crawford discovered that light entering different parts of the pupil of the eye did not give rise to equal retinal response, that is, it was found that the retina was differentially directionally sensitive to light. For such a phenomenon to occur, two conditions are necessary:

First, some component or components must be differentially directionally sensitive, and second, these components must be aligned with some degree of uniformity.

By studying directional sensitivity patterns it is possible to obtain insight into the state of organization and orientation of that which is aligned. The Stiles-Crawford effect is largely associated with retinal cone vision, but in one case, reported by Flamant and Stiles,[4] where the peripheral retina was interpreted as being tilted by the authors, a

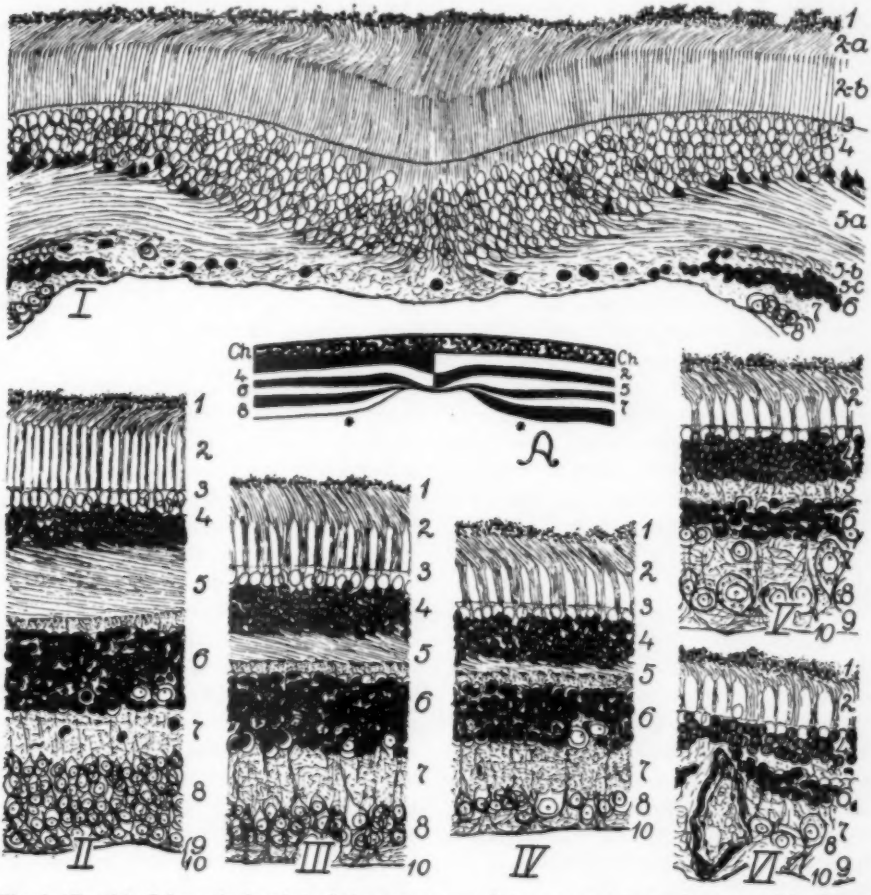


Fig. 1 (Enoch). Schematic drawing of the appearance of the receptors on a microscope slide. One can conceive that such a distribution might occur *in vivo*. (Courtesy, University of Chicago Press, from *The Retina* by S. Polyak.)

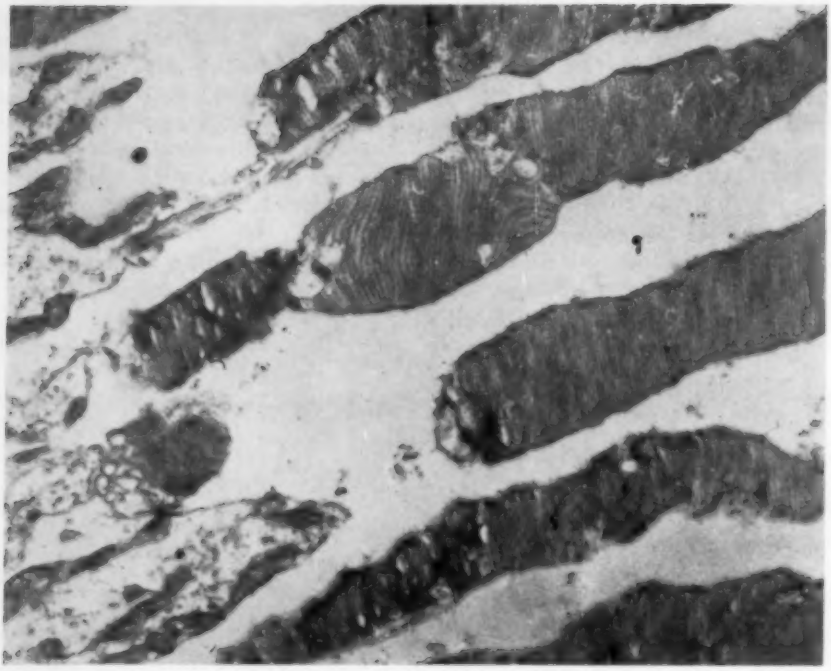


Fig. 2 (Enoch). Retinal rod outer segments of the adult mouse. Note the disturbed lamina in the one receptor unit. (Courtesy Dr. A. Cohen, Department of Anatomy, Washington University Medical School.)

change in directional sensitivity in rods was noted. Ordinarily however, one may make use of the fact that rods show virtually no change in directional sensitivity, for light entering different parts of the pupillary aperture, to aid in separating retinal rod and cone function. A typical normal foveal directional sensitivity pattern is shown in Figure 3. Relative sensitivity is plotted as a function of angle of incidence of light at the retina. The direction of maximum sensitivity for this subject varies slightly from the center of the entrance pupil. The magnitude of the effect is ordinarily slightly less than one logarithmic unit. The function should be thought of as a figure of revolution in order to consider all meridians in the pupil.

If the alignment of the oriented components is disturbed, certain things may be expected to occur. First, if the direction of maximum sensitivity of these components is such that they are not directed at the center of the entrance pupil, less light will be absorbed by these receptors. That is, one may think of the pattern shown in Figure 3 as being tilted more on its side. It is well known that acuity is dependent upon the amount of light absorbed.[5] Because of the nature of this function at higher levels of luminance, it is theoretically possible to isolate that part of visual loss contributed by this factor. Secondly, under certain specified stimulus conditions one would anticipate that these receptors, with their directionally oriented components aligned as in the first instance, would absorb greater amounts of stray light.[6] Stray light acts to reduce constrast, which, in turn, affects acuity.

These two types of disturbance may be

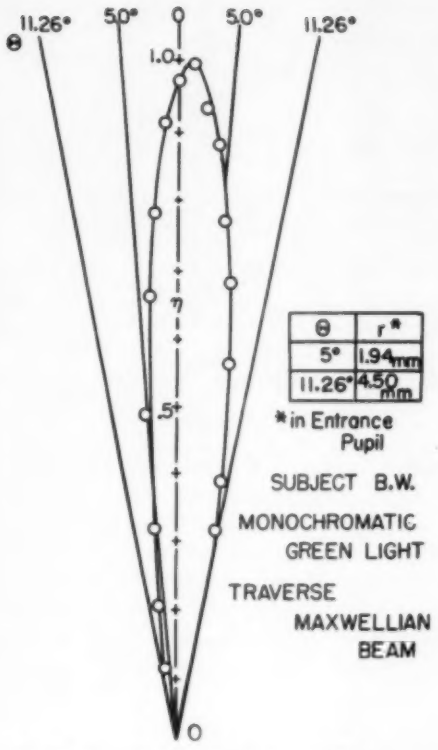


Fig. 3 (Enoch). Relative luminous efficiency as a function of retinal angle of incidence. This is a typical normal Stiles-Crawford function. Equivalent entrance pupil radii are presented. η represents relative directional sensitivity.

independent of total receptor orientation, that is the orientation of some component within the cell may be disturbed (as in Figure 2, or see reference[7]), as contrasted with the orientation of the cell itself. Of course, a large number of cells would have to be involved if function is to be impaired. If the orientation of the receptor cells is disturbed, the above two factors plus two additional aspects must be considered. First, if one were to consider the case of light energy incident normally at the retina, it might be anticipated that fewer receptors would be stimulated if these receptors were aligned parallel to the incident light, than if the receptors were oriented at an angle to the incident energy (fig. 1). The effect is obviously to reduce the retinal "grain." In other words, the resolution of a receptor system is dependent upon the area (or angle) covered (subtended) by each of the individual elements. Oblique incidence or oblique orientation therefore precludes fine resolution. It also follows that if all fibers were uniformly tilted, acuity would vary as a function of the meridian tested. Campbell[8] has recently shown that for oblique incidence of light at the retina in a normal eye, visual acuity drops by a factor of eight times (for example, 20/20 to 20/160) as the test beam moves from the center to the edge of the entrance pupil. This loss in acuity is manifested for a grid target oriented perpendicular to the direction of beam movement.

A second consideration was revealed as part of a recent investigation* of model retinal receptors studied at microwave frequencies.[9] The models employed (fig. 4) were only of the ellipsoid portion of the receptor cells. In brief, it was found that if the axes (center lines) of the models were parallel, that is, $\beta = 0°$, there was little interaction between receptor elements for virtually all angles of incidence of energy. The axes of the receptors were rotated relative to the optic axis of the microwave system, and were placed at various positions and planes in

* This phase of the program was sponsored by a contract between the Air Force Office of Scientific Research and the Ohio State University Research Foundation (contract No. AF 18 (603)—63).

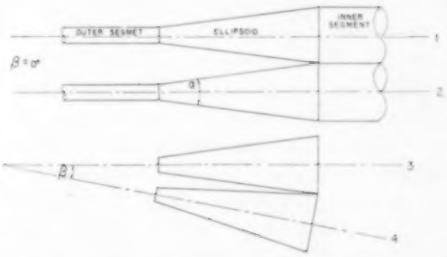
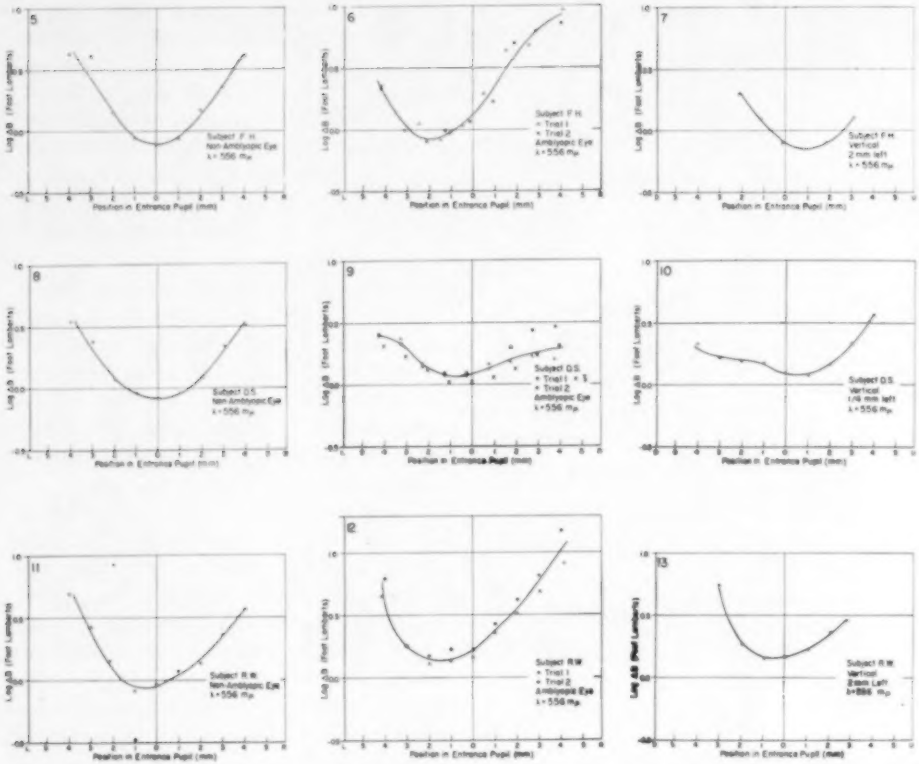


Fig. 4 (Enoch). Illustration showing the nature of the experimental arrangement in the microwave study.



Figs. 5 to 13 (Enoch). Foveal threshold determinations for light entering different points in the entrance pupil of the eye. (Figure numbers appear in the upper left-hand corner of each graph.)

the diffraction pattern. This finding implies relative independence or integrity of the energy path once the energy enters the ellipsoid. The maximum change in recorded power absorbed due to the presence of an additional receptor was 0.2 log unit.

However, if β was not equal to zero, the interaction between elements increased (note β maximum is equal to α). This interaction seemed greatest when $\beta = \alpha$ or when the surfaces were parallel. For this to occur, the surfaces did not have to be in contact—rather merely contiguous to each other. If separated by one or two wavelengths, or less, this effect was noted. Interactions up to about 1.0 log unit were found.

In other words if the ellipsoids or outer segments of neighboring receptors come into close relationship with each other, the energy within the receptors interacts to a considerable degree causing a loss in the integrity of the individual light paths and hence in visual resolution. The causes of this finding are complex. This then is another mechanism giving rise to reduced visual acuity, that is., close or actual contact of neighboring ellipsoids or outer segments. These interaction phenomena are also known as frustrated total reflection. Recent work in the field of fiber optics[10–12] tends to support these findings.

Thus, at least four mechanisms are suggested which will tend to reduce acuity if that which is oriented in the fovea is dis-

TABLE 1

DATA RELATED TO THE TEST PROCEDURES

Subject	Age	Eye	Corrected V.A.	Refraction	Directional Sensitivity	Direction of Maximum Sensitivity	
						Horiz.	Vert.
F. H.	35	O.D.	20/25	$-1.25 = -0.25 \times 105$	Simple tilt	5.00°L	2.50°U
		O.S.	20/15	$-1.50 = 0$	Normal	0	0
D. S.	11	O.D.	20/60	$+2.00 = 0$	General Malorientation	A	A
		O.S.	20/15	$+0.50 = 0$	Normal	0	0
R. W.	19	O.D.	20/60	$-2.25 = -0.75 \times 175$	Simple tilt + Reduced Sensitivity	5.00°L	2.00°D
		O.S.	20/20	$+1.25 = 0$	Normal	1.25°L	1.25°U

A = Not applicable.
Note: 1 mm. in Entrance Pupil = 2.50° change in angle of incidence.

turbed. On the basis of these data one may hypothesize that a certain percentage of individuals with reduced visual acuity or amblyopia due to either physiological or pathological causes would be expected to have noncustomary directional sensitivity patterns as regards form or orientation. One could consider such individuals as manifesting receptor amblyopia.

EXPERIMENTAL

The data presented below (with the exception of patient P. B.) have been presented in a more complete form elsewhere.[13, 14] Stiles-Crawford type data may be obtained using any of several mutually satisfactory methods. In the three cases whose data are described in Figures 5 to 13, a contrast threshold (ΔB) was determined by an ascending method of limits.[14] In the case seen in Figure 14, a bipartite field was matched in luminance by a method of adjustment.[13] In that instance relative sensitivity is plotted. It will be remembered that a measure of sensitivity may be determined by plotting the inverse of threshold (although this was not the method used in this instance).

If a parallel plane wave having as its origin a point source at infinity is incident at the cornea, the rays are refracted to form a theoretical point image (of the point object at infinity) on the retina in an emmetropic eye. The more peripheral the ray striking the cornea, the more the refracted ray striking the retina departs from normality to the surface. In testing for the Stiles-Crawford effect, one in effect samples across a plane wave incident at the cornea and measures by some means the excitation occurring in the retinal plane. Thus, the data below are expressed in terms of the radial distance of incidence of the test beam in the entrance pupil of the eye. This may be transformed into angle of incidence at the retina (fig. 3) in terms of the simple relationship given in Table 1.

Three types of data are obtainable from the functions observed in Figures 5 to 13.

1. Directional sensitivity functions of the

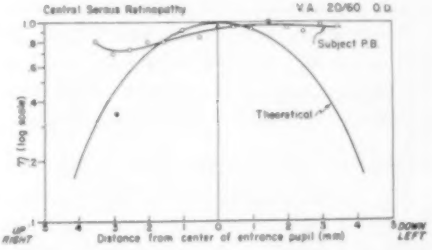


Fig. 14 (Enoch). Directional sensitivity (η) data of subject P. B. compared with a theoretical distribution which gives a good approximation to data obtained from a normal eye.

central fovea. From these one may deduce information as regards organization and orientation of that which is oriented.

2. Comparative sensitivity of the two eyes. It was in order to obtain these types of data that a contrast threshold (ΔB) technique was employed. Properly one should integrate the functions for a given pupil size in order to obtain this information. A first estimate is derivable by comparing ΔB values in the two eyes at $r = o$.

3. Comparative sensitivity of the two retinae. These types of data are obtained by comparing ΔB values at r (maximum sensitivity) in the two eyes. If these ΔB values are not equal, no statement may be made on the basis of this technique as to where the loss in sensitivity has occurred in the total pathway.

The hypothesis stated above was first tested on a group of individuals who had one normal eye, and one amblyopic eye. In order to avoid problems of faulty fixation, maximal visual loss considered was 20/60. All patients had good central monocular fixation in both eyes, and no evident pathology.

Six individuals were tested. These tests required a minimum of twenty hours of the patient's time in addition to the routine refractive techniques and tests for fixation. Patients were compensated in part. Obviously this cannot be considered as a technique for routine clinical testing at this time. In the amblyopic eye of the six individuals tested, two manifested simple tilt of that which is oriented, two manifested general malorientation, one manifested simple tilt plus generally reduced sensitivity, and one exhibited no significant change in either directional sensitivity or retinal sensitivity.

Simple tilt of that which is oriented

This is characterized by a significant shift in the direction of maximum sensitivity from the center of the entrance pupil. The amblyopic eye is less sensitive to light than the normal eye (comparison of ΔB values at $r = 0$), and the sensitivity of the two retinas is approximately the same (comparison of ΔB values at r (maximum sensitivity)).

An example of this type of manifestation is seen in Figures 5, 6, and 7. Note that "vertical" applies only to the amblyopic eye since there seemed little point in reproducing the vertical functions in normal eyes. However, the direction of maximum sensitivity in the vertical meridian (measured through the point of maximum sensitivity in the horizontal meridian) is shown in Table 1. Subject F. H., a highly trained visual experimenter, had noticed his visual acuity in his right eye (20/15 to 20/25) has been slowly dropping during the last few years.

One may raise a question as to magnitude of tilt necessary for loss in acuity? There are several approaches to this question. A statistical approach has been discussed elsewhere.[14] The technique of Campbell[8] provides a superior method for evaluating at least one aspect of the visual loss.

General malorientation of that which is oriented

General malorientation is characterized by a general flattening (causing a basic change in the nature) of the directional sensitivity pattern. It is my belief that this represents n groups of receptors oriented in different manners.[14] ΔB at $r = 0$ is not equal in the two eyes, and ΔB at r (maximum sensitivity) would be expected to be somewhat greater in the amblyopic eye.

Subject D. S. (figs. 8, 9, and 10) manifests this type of condition. This subject had presented himself with 20/200 vision in his amblyopic eye. Routine training procedures had increased his visual acuity to 20/60 in that eye, but no further improvement could be obtained. It was at this point that the patient was seen. This patient tended to suppress this amblyopic eye during binocular testing, and manifested an eccentricity of fixation of 2^{Δ} B.O. during binocular fixation. During monocular testing there was no suppression and no apparent eccentricity of fixation.

Reduced sensitivity independent of orientation changes

This condition is characterized by normal orientation of that which is oriented. However, Δ B at r (maximum sensitivity) in the amblyopic eye will be greater than that in the normal eye.

No case of this type was found. However, subject R. W. shows a combination of simple tilt and reduced sensitivity (figs. 11, 12, and 13). His simple tilt is of the order of magnitude of that manifested by subject F. H., but in Table 1 note the difference in acuity in the amblyopic eyes of the two subjects. In the case of subject R. W., Δ B at r (maximum sensitivity) is greater in his amblyopic eye than in his normal eye.

As was noted above, one subject, with visual acuity in his amblyopic eye of 20/40, was tested who showed no change in the characteristics measured in this experimental program. He reported fading of the adaptation field during testing of the amblyopic eye, and had to blink continuously in order to maintain the field. This presented no real impediment in testing.

It is obvious that what the author is seeking to achieve is the development of an etiological breakdown of amblyopias. On the basis of the work presented above, it is tentatively possible to classify three types of amblyopia by this technique, two of which would seem to be receptor in origin. Further, amblyopias exist (as anticipated) which cannot be classified by this technique. Much further study is required to validate these findings, and to extend the etiologic breakdown.

These techniques also have considerable application to pathological processes. A preliminary venture in this direction is shown in Figures 14 and 15. Patient P. B.* exhibited central serous retinopathy. Figure 15 shows the nature of the lesion and the meridian tested. At the time of testing she manifested 20/60 visual acuity. Since as a laboratory technician she did routine colorimetric determinations, she could be considered at least a partially trained subject. There was no problem concerning the stability, or the central nature of her fixation. She did exhibit some metamorphopsia. Figure 14 represents a determination of her central retinal directional sensitivity pattern. This pattern falls in the general malorientation category described above. A typical normal pattern is superimposed for purposes of comparison.

It should be emphasized that data of these types may be obtained from any point in the retina. The central region, however, offers certain obvious experimental advantages.

Clinical instrument

Before these techniques can have real clinical value, it will be necessary to find means and methods to simplify the necessary apparatus, and to reduce the test period significantly. The device described below is considered a first step in this direction. This instrument will be suggested largely for a screening function because various factors tend to limit its accuracy to a certain degree. These limitations will be noted at the appropriate point in the text below. A more sophisticated instrument[14] is available at this institution for detailed study. However, the simplicity, and compactness of the unit de-

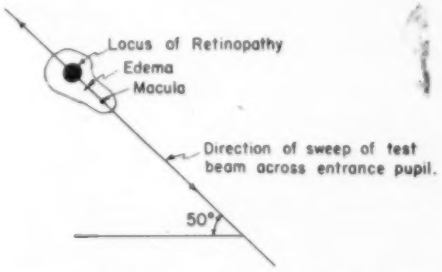


Fig. 15 (Enoch). Schematic diagram showing the nature of the pathology, and the direction of the sweep across the entrance pupil of the eye of subject P. B. in order to obtain the data shown in Figure 14.

* I wish to thank Dr. William Havener for referring this patient to my attention.

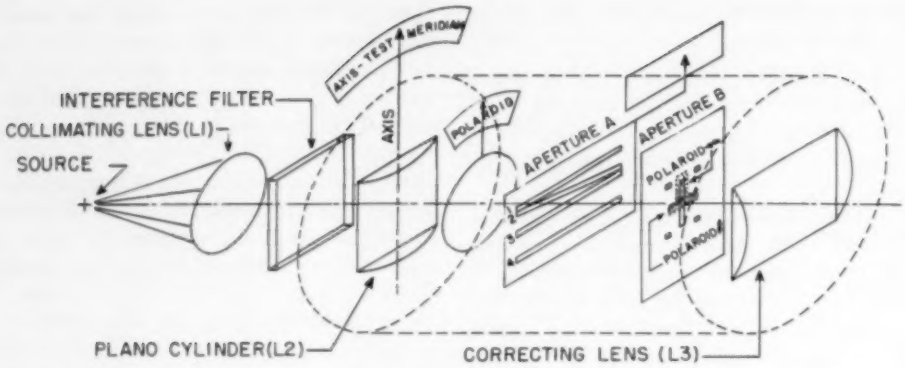


Fig. 16 (Enoch). Schematic drawing of the prototype clinical Stiles-Crawford apparatus.

scribed below recommends it for clinical studies.

A Stiles-Crawford apparatus may be successfully designed if one utilizes the features of a cylindrical lens. The device need have only one lens instead of the two described below if one does not have a desire to use interference filters in order to produce monochromatic light. That is, the collimating lens (L1 in Figure 16) may be combined with the plane cylinder (L2) in a spherocylindrical lens.

Figure 16 is a schematic drawing of the instrument. An aperture covering a uniformly illuminated piece of ground glass acts as the light source. It is suggested that the aperture subtend about one half degree visual angle at the eye. This light is collimated by achromatic lens L1, and is made essentially monochromatic by an interference filter.

It is necessary to make the remainder of the instrument rotateable about the optic axis in order to allow testing in any meridian at the eye. This is indicated by the large cylindircal figure surrounding the remaining elements. A plano-cylindrical lens (L2), for example, +10D., is employed to focus the beam in the entrance pupil of the eye. For clinical purposes, a figure of three millimeters to the rear of the apex of the front surface of the cornea may be used as entrance pupil. The remainder of the units must be crowded as close as possible to L2. The order of these elements is exchangeable with the exception of the correcting lens (L3). A variable polaroid is included to allow matching of brightness at different settings of aperture A. Aperture A selectively illuminates parts of the line image in the entrance pupil of the eye, allowing determination of the Stiles-Crawford effect. Aperture B limits the extent of the visual field in one meridian, and allows selection of the retinal area to be tested (peripheral or central). Note that fixed polaroids are attached to aperture A or aperture B crossed at 90 degrees in order to allow measurement. In order to correct errors of refraction, or to correct aberrations, or to vary alignment, plane cylindrical (trial set) lenses (L3) may be placed as the last element in the system. These are aligned with their axis 90 degrees from the axis setting of lens L2.

Figure 17-A and B provides a somewhat better picture of the manner in which the instrument works. From Figure 17-A one can see the manner in which the image in the entrance pupil is formed. This image would be a line perpendicular to the plane of the page. In Figure 17-B it is seen that aperture A allows only parts of that line image to pass. From Figure 17-A one notes that aperture B limits the extent of the field (bar image on

retina). For central retinal testing a one degree extent is suggested. For parafoveal and peripheral testing, one merely laterally displaces the aperture in plate B, while at the same time providing a fixation point. Obviously any number of other arrangements may be made. In Figure 17-B if one looks at aperture A one notes that four (or any number of) separate beams are allowed to pass. Apertures 1 and 4 are designed to aid in centration of the pattern in the entrance pupil. Double lateral apertures may be cut in aperture plate B to avoid interference with the test pattern in the retinal plane. These also serve as a useful device in fore and aft positioning of the patient. That is, if the line is not imaged properly in the entrance pupil, the two beams will not exactly cross, and by lateral head movement (for the setup shown) one or the other of the retinal images of these centration beams will disappear first. If the eye is correctly positioned both will disappear together with lateral movement. The central testing array shown in Figure 18 will clarify this point. If fore and aft placement is correct, since these apertures are placed near the edge of the dilated pupil, disappearance or vignetting of a pair of the retinal images will signify vertical or horizontal movement of the entrance pupil.

In the arrangement shown (central testing, Figure 18) beam 3 is centered on axis and is oppositely polarized from the probing beam number 2. Beams 2 and 3 act in a manner similar to a Scheiner's double aperture disc. Lens L3 is used to vary the separations of, or move these images at the will of the experimenter. With lateral movement of aperture A (Figure 16), beam 2 is swept across one half of the entrance pupil enabling measurement of the Stiles-Crawford effect. The separation of the two images of beams 2 and 3 may be kept constant on the retina by varying lens L3, hence overcoming the effects of errors of refraction and of spherical aberration. This causes some displacement of beam two in the entrance pupil. For peripheral or parafoveal testing an arrangement such as is shown under "parafoveal testing" in Figure 18 is recommended. It would be necessary to invert aperture A midway through the examination in order to obtain a complete set of data. While for central testing any meridian may be studied, peripheral testing is limited to the meridian oriented perpendicular to the radial direction of displacement. For screening, the direction

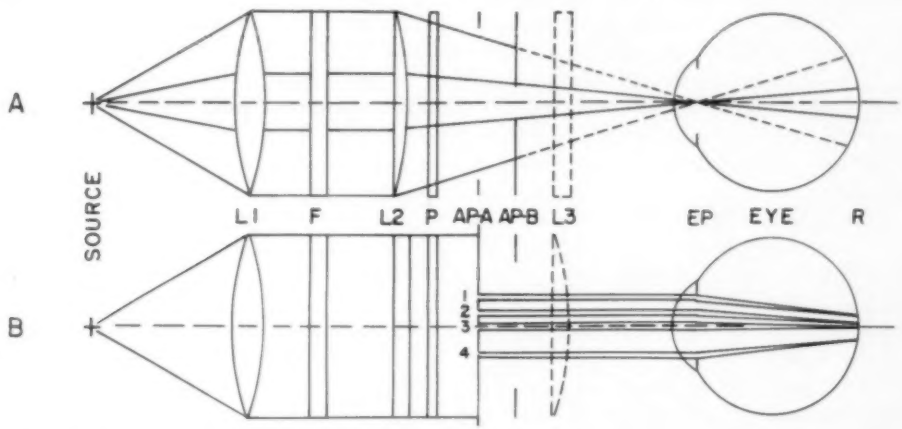


Fig. 17 (Enoch). Ray trace diagram of the clinical Stiles-Crawford apparatus. The letter designations correspond to the listed elements in Figue 16. EP = entrance pupil, R = retina. (A) The axis of lens L2 is perpendicular to the plane of the page. (B) The axis of lens L2 lies in the plane of the page.

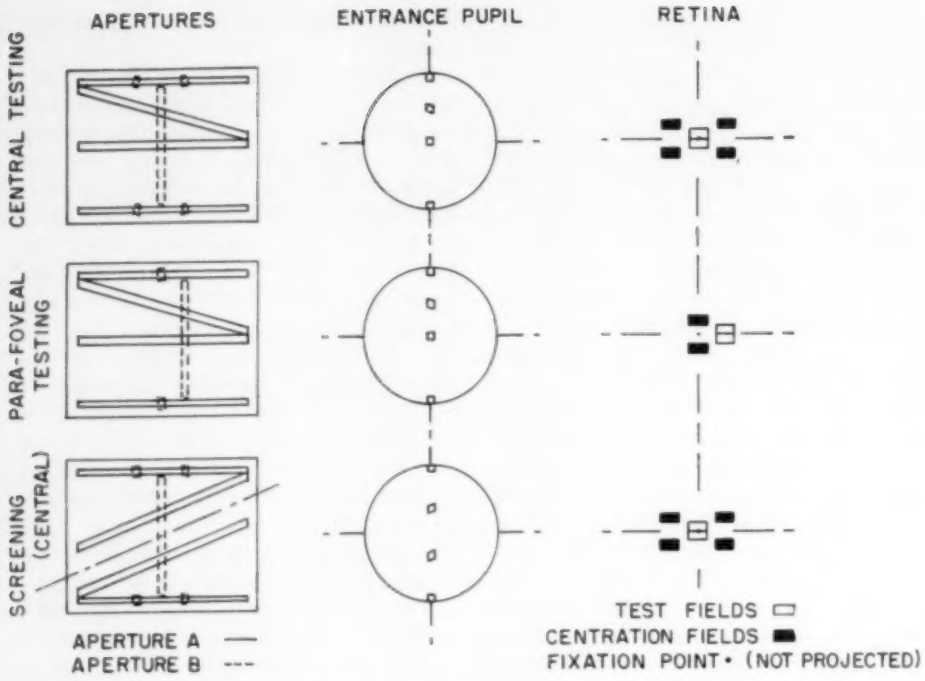


Fig. 18 (Enoch). Sample apertures to be used in various testing situations. The top and bottom lines of apertures A have been shortened in length to have space.

of maximum sensitivity for central vision may be readily determined by using the arrangement shown under "screening (central)" in Figure 18. Here the polaroids may be removed from aperture B, and a match is made by moving aperture A laterally until the two fields appear equal. The direction of maximum sensitivity would be the bisector. Obviously any number of arrangements may be introduced.

It is apparent that the same device, with manipulation of apertures A and B and filters, may be used to measure spherical and chromatic aberration, and to determine the achromatic axis of the eye. Because the apertures (aperture plate B) which limit the ends of the field are not imaged at the retina, somewhat blurred imagery should result at those points. In order to produce a bipartite field for testing some blur is tolerated in the pattern. This influences measurements slightly.[15] Another limitation of the instrument is the fact that it gives no measure of the sensitivity of the eye or the comparative sensitivity of the retinae. The former, i.e., sensitivity of the eye may be measured readily in any number of ways. As noted above, patients must have good central fixation and be held rigidly during testing. Since the Stiles-Crawford effect is a physical phenomenon largely independent of luminance,[16-18] control of this factor, once set, is not too critical except in certain specified test circumstances.

Summary

Obviously the discussion above introduces a program of research. Many phases need further investigation. It is hoped much basic information may be derived concerning retinal mechanisms, and perhaps clinical procedures will be developed, enabling better

classification of conditions, and more accurate prognosis in cases of reduced vision.

A theory has been developed relating possible disturbances in retinal receptor, and receptor component orientation to visual function. It was predicted that such disturbances would lead to amblyopia. In five of six tested cases with amblyopic eyes, it was found that receptor orientation was disturbed. Three types of amblyopia may be classified using the techniques employed, of which two are probably receptor in origin. The techniques employed were applied to one case with central serous retinopathy. This individual was also found to manifest disturbed receptor orientation. Since required test time is a major problem, a prototype simplified clinical or screening instrument has been described which, it is felt, will make possible more rapid screening and testing of patients.

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Discussion

Dr. Burian (Iowa City, Iowa):

Dr. Enoch has presented to us a study which is of greatest interest to everyone interested in retinal physiology and retinal pathology. It is of particular interest also to those who concern themselves with the so-called amblyopia ex anopsia or strabismic amblyopia.

The Stiles-Crawford effect is a well-established phenomenon and I shall not raise the question here to what extent a tipping of the crystalline lens or a large angle kappa may influence the measurements obtained by Dr. Enoch. But I do wish to bring up one point. Dr. Enoch has reported on cases in which he found what he interpreted to be a "simple tilt" or a "general malorientation" of visual cells in the retina. Yet the acuity of the amblyopic eye of these patients could be improved by ordinary means. In one case occlusion of the fixating eye improved the vision of the amblyopic eye from 20/200 to 20/60, but not beyond this point. This observation suggests two thoughts. One is that in these patients in addition to the malorientation of the sensory cells of the retina, another factor must be operative in producing the amblyopia, for instance some sort of sup-

pression or inhibition, since it cannot be assumed that the occlusion of the fixating eye resulted in a reorientation of the retinal elements of the amblyopic eye.

The other thought is this: In the past I have assumed, as have others, that in patients in whom intensive attempts at re-educating the visual acuity of the amblyopic eye were not successful in improving the acuity of that eye beyond a certain level, we were faced with an amblyopia of pathologic origin, even though we were unable to discover any fundus anomalies with the ophthalmoscope. Ever since the work of Bangerter and Cüppers became known, a number of my friends interested in the amblyopia problem have pointed out to me that there was no such thing as a pathologic strabismic amblyopia and that my assumption was simply based on my inability to diagnose small degrees of eccentric fixation.

Dr. Enoch's work opens up the possibility of reviving the concept of a pathologic strabismic amblyopia. The question arises then, how can a malorientation of the retinal elements occur, such as Dr. Enoch believes to be present? Many years ago, stimulated by a case of what we then called pachymeningitis hemorrhagica, the eyes of which I had an opportunity to study histologically. I examined a number of eyes of newborn children ophthalmoscopically and was struck by the frequency with which retinal hemorrhages, often rather large and numerous ones, were seen in these infants. These hemorrhages disappeared without leaving a trace and had no appreciable effect on the function of the eyes. It is quite conceivable that such hemorrhages, if they occurred at the posterior pole of the eye, while not affecting the eye grossly might produce a disarrangement in the orientation of the sensory retinal element and result eventually in such phenomena as the ones which Dr. Enoch has described for us.

I have just one question to ask Dr. Enoch. It was not clear to me why Dr. Enoch felt that in his last case in which the patient had to blink to "restore" the brightness of the adapting field, the seat of the lesion was central to the receptors.

I wish again to congratulate Dr. Enoch on his excellent presentation of a beautiful piece of work.

DR. ENOCH (closing): I certainly want to thank Dr. Burian for his kind remarks. I shall endeavor to answer the questions raised as best I can.

Any factor which will introduce obliquity of incidence of the light, or of the receptors (relative to each other) should result in the findings described above to some degree.

There is no doubt that the ambylopia of subject D.S. consisted of more than one factor. The same may be said of subject R.W. This is why it is important to develop a more complete etiology of amblyopia, in order that we may predict the results of training, and to direct our attention to the specific needs of the patient. Where training or other procedures are not yet possible, such a breakdown of amblyopias will serve to guide our research. This study is but a first step in such a breakdown.

In the case of the subject who restored field brightness by blinking, one cannot say that the fault lay in the photochemical response mechanisms of his receptors. From our knowledge of dark adaptation, a brief blink is not sufficient to restore photochemical concentrations. Further, the tests completed in this series show that retinal response is normal (as defined above). Rather, it would seem that this defect would be in the excitation transmission system or in the interpretive centers. In terms of the tests performed, one cannot tell at this time at which level this defect is mediated.

THE EFFECT OF TWIN FLASHES AND OF REPETITIVE LIGHT STIMULI ON THE HUMAN ELECTRORETINOGRAM*

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The size and shape of the electric responses of the retina depend, aside from physical factors of the stimulus and technical factors of recording, on biologic factors, the state of the retina. Illumination of the retina by a flash of light produces changes in the state of the retina which last beyond the duration of the flash. Thus twin flashes, and the use of repetitive light stimuli, offer a

* From the Department of Ophthalmology, College of Medicine, State University of Iowa. This work was performed during the summer of 1956 during the tenure of one of us (B. E. S.) of a College of Medicine, State University of Iowa Summer Student Fellowship, and was supported by Grant B-149 (C-3) of the National Institute of Neurologic Diseases and Blindness, National Institutes of Health, Bethesda, Maryland. Presented at the Mid-Winter National Meeting of the Association for Research in Ophthalmology, Edgewater Park, Mississippi, February 20, 1959.

tool for the study of the effect of photic stimulation on the retina.

The effect of twin flashes on the retina of animals has been investigated by a number of physiologists. Studies in man were done by Dodt[1] and Mahneke.[2] Repetitive stimuli have proved useful not only in physiologic experiments but also in clinical electroretinography.

The present report deals with a study in which we employed twin flashes of four intensity levels, but equal for each set of flashes, separated by 15 to 150 msec. intervals, and repetitive stimuli with comparable intervals.

Techniques and Procedure

Experiments were performed on five subjects, students between the ages of 20 and 24 years, without significant ocular anomalies.

The light source was provided by a PS-2 Grass Photo Stimulator: Intensities 1 (I_1), 4 (I_4), and 16 (I_{16}) were employed. In addition, a fourth, low intensity was obtained by a neutral filter consisting of an exposed X-ray film, giving a reduction by two log units. It was used in conjunction with I_1 (I_1F_2). The photo stimulator could be operated manually or automatically by a Hunter Timer, delivering one flash every four seconds.

The active electrode was a Burian-Allen[3] speculum electrode; the indifferent electrode, a silver cup electrode, was fastened to the supraorbital area of the eye to be tested; grounding was provided by two electrodes, one fastened to each earlobe of the subject.

Recording was done simultaneously with a Grass 4-channel EEG pen-writer and a Dumont oscillograph and Grass camera. The paper and film records were correlated by a lapse time indicator (Arnott[4]). The details of the experimental arrangement will be described in a forthcoming publication.

The experimental procedure was as follows. After the subject was prepared, he was light adapted for five minutes to the light reflected from a bowl painted with non-glossy white paint. He was then dark adapted for 10 minutes and the automatic light flashes were started. The intensity remained unchanged during each experimental session. To maintain a steady level of adaptation, a flash was delivered automatically every four seconds throughout the whole session, except at the end when repetitive stimuli were used. After the first minute of delivering the flashes, recording of single flashes were made for one-half minute. At the 12th minute double flashes of 15 msec. flash interval were delivered for one-half minute, followed again by one-half minute of single flashes. The procedure was repeated in exactly the same fashion with double flashes of 20, 30, 40, 60, 70, 80, 90, 100, 120, 150 msec. intervals. At the conclusion of this series repetitive light stimuli with frequencies of 1, 2, 4, 6, 7, 8, 10, 12, 15, and 20 stimuli per second were delivered.

A few exploratory experiments were discarded. One experimental series on each eye of each subject was utilized for this report. For each stimulus situation in each series three electroretinograms were measured and the measurements averaged. These averages were then again averaged for the whole group.

Analysis and discussion of results

The results will be analyzed under four headings: (1) The single flash electroretinogram; (2) the effect of twin flashes on the first electroretinogram; (3) the second electroretinogram with twin flashes; (4) the electroretinogram with repetitive flashes.

1. The single flash electroretinogram

While the data obtained with single flashes do not add essentially new information, they furnish a clear and consistent picture of the differences in the size and shape of human electroretinograms obtained with stimulating flashes of four intensity levels.

In most of our electroretinograms the four typical components of the electroretinogram resulting from flashes of brief duration, a

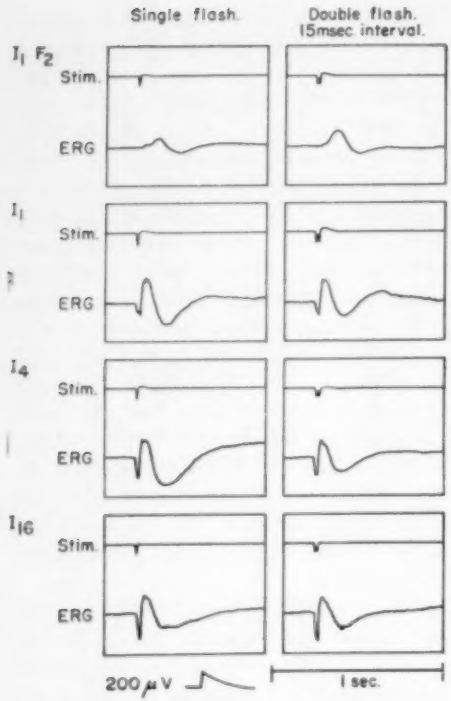

Fig. 1 (Burian and Spivey). Electroretinograms obtained with single flashes of four stimulus intensity levels (left hand column) and with double flashes having a 15-msec. interval (right hand column). Note the small positive hump early in the response with I_1F_2 and the difference in size of the responses with I_1F_2 of the single flash and the double flashes. Note also the difference in shape of the electroretinograms with single and double flashes at the higher intensities, demonstrating the reduction in the scotopic component with the double flashes.

double a-wave and a double b-wave, were clearly discernible, with the exception of the electroretinograms produced by I_1F_2 flashes which never gave an a-wave (fig. 1). In some of the electroretinograms the positive elevation was represented by a plateau rather than by two distinct peaks, but in the majority of them two peaks could readily be measured.

We shall speak of the four components of the electroretinogram as of the first and second negative and positive elevations and designate them as a_1, a_2, b_1, and b_2. Such a designation, being purely descriptive, avoids any theoretic implications and should be preferable even to the designation a_p, a_s, b_p and b_s, suggested by Johnson[5] to avoid the existing terminologic confusion, where the subscripts p and s stand for photopic and scotopic, respectively.

The summary of the averages of the measurements obtained (table 1, fig. 2) permits the following conclusions.*

1. Stimulation with low intensity (I_1F_2) resulted in electroretinograms from which the a-wave was always absent. There was, however, in all of the electroretinograms a small first positive elevation which had a peak time of 65 msec. which we believe to correspond to the b_1-wave of the electroretinograms obtained with more intense flashes (fig. 1).

2. With intensity I_1 an a_1 and a_2-wave, and a b_1 and b_2 wave were obtained, with the first negative and positive components being *smaller* than the second ones.

3. With intensities I_4 and I_{16} the situation was reversed, the first negative and positive deviations being *larger* than the second ones.

4. The absolute values of the b_1- and b_2-waves were somewhat smaller for I_{16} than for I_4.

5. The peak times of a_1 and of a_2 were the same for all intensities, whereas with the higher intensities there was a reduction in the peak time of b_1 and especially of b_2, the peak time of b_2 for I_{16} being only one half of that for I_1F_2.

These findings summarize and confirm the results obtained with many different methods in this laboratory and in others. They clearly support the assumption that a_1 and b_1 are essentially expressions of the *photopic* process: at a given level of dark adaptation they are smaller for low stimulus intensities and larger for high stimulus intensities than their counterparts a_2 and b_2. These latter com-

* In evaluating the mean deviations it should be understood that they relate to the differences between subjects. For measurements in the individual subjects the deviations were generally much smaller.

TABLE 1

AVERAGE AMPLITUDES (A) AND PEAK TIMES (t_p) OF THE FOUR COMPONENTS OF THE ELECTRORETINOGRAM WITH SINGLE FLASHES OF FOUR INTENSITY LEVELS

	a_1		a_2		b_1		b_2	
	A μV	t_p msec	A μV	t_p msec	A μV	t_p msec	A μV	t_p msec
I_1F_2	—	—	—	—	33 ± 3	65 ± 7	123 ± 14	110 ± 4
I_1	97 ± 19	13 ± .1	107 ± 19	22 ± .1	373 ± 24	45 ± 4	403 ± 16	61 ± 3
I_4	230 ± 18	15 ± 2	233 ± 8	20 ± 1	479 ± 24	43 ± 2	443 ± 13	60 ± 2
I_{16}	276 ± 17	18 ± 2	263 ± 29	22 ± 2	456 ± 47	43 ± 3	433 ± 63	53 ± 5

ponents would then be the expressions of the *scotopic* process.

The fact that b_1 and b_2 have smaller absolute values for I_{16} than for I_4 bears out an old observation made in this laboratory (Burian[6]) that supramaximal stimuli tend to reduce the b-wave, whereas the a-wave is not so affected.*

There is no difference of opinion regarding the existence of a double b-wave, or for that matter of a double a-wave, but it is not clear whether the first positive elevation can be equated under all circumstances with the so-called x-wave. Auerbach and Burian[7] assumed this to be the case. Schubert[8] summarily rejected their interpretation, stating that the differential construction of the electroretinogram suggested by them was wrong because of faulty measurement (Ausmessung) of the x-wave.

Best[9] has confirmed the findings of Auerbach and Burian, using red stimuli of increasing intensity, and following up, as they did, the behavior of the electroretinogram throughout dark adaptation with a white stimulus light. While Best is, therefore, inclined to agree that the first positive elevation is in fact the x-wave, he points out correctly that the continued increase in size of the first positive elevation during dark adaptation, and especially the rapid change in rate of this increase after the 11th minute, would indicate that both the photopic and the scotopic activity contribute to the appearance of the x-wave. However, if this explanation is correct, it introduces another complication, namely that we must assume the existence of quick acting scotopic responses in addition to the well known slow acting ones.

One wonders what elements might be giving this response. Also, it will be hard to answer the question, when the first positive ele-

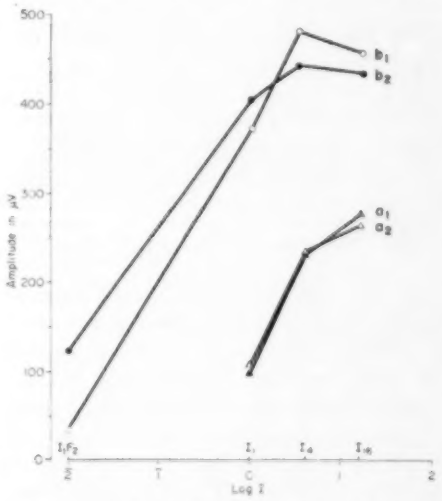

Fig. 2 (Burian and Spivey). Plot of average amplitudes of the components of the single flash electroretinograms at four intensity levels of the stimulus. Note the inversion of the quantitative relationship of the components of the I_4 and I_{16} level, compared to levels I_1F_2 and I_1.

* It must be pointed out that at the time this observation was made we measured the b-wave from the iso-electric line, not from the trough of the a-wave. When it is done in the latter fashion—and this has now been our practice for quite some years —the effect is not invariably found.

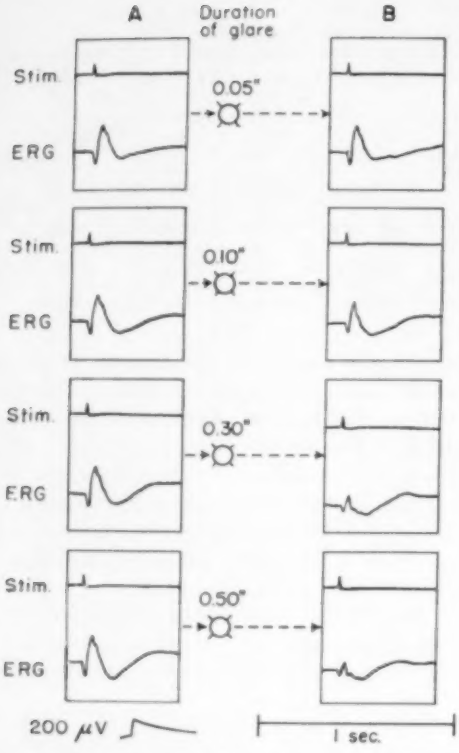

Fig. 3 (Burian and Spivey). Tracings demonstrating effect of light adapting flashes of short duration on electroretinograms of normal dark-adapted human eye. Column A: last electroretinogram prior to flash. Column B: first electroretinogram following the flash. Note progressive reduction of b_2-wave, as well as of a- and b_1-waves, with corresponding progressively "photopic" appearance of electroretinogram and "isolation of x-wave."

vation becomes an "x-wave," unless it is true, as Armington[10] thinks, that the x-wave is a specific "red" response, not an over-all photopic response. Consider for example such an experiment as the one reported by Burian,[11] in which dark adaptation was interrupted by light adapting flashes of varying duration (0.05" to 0.5"). The first electroretinogram following the bright flash showed a definite change in size and shape compared with the last electroretinogram preceding the flash. With increasing duration of the light adapting flash the "photopic" character of the electroretinogram following it became increasingly prominent (fig. 3). Has the b_1-wave already become an "x-wave" with the light adapting flash of 0.1" or only with the 0.3" flash?

Regardless of the solution to this problem, there is ample evidence that the b-wave is composed essentially of two peaks and that these peaks can be differentially affected, depending on the condition and state of adaptation of the eye and on the parameters of the stimulating light.

2. Effect of twin stimuli on the first electroretinogram

At no intensity could a second electroretinogram be elicited by twin flashes with a 15 and 20 msec. delay interval; very much

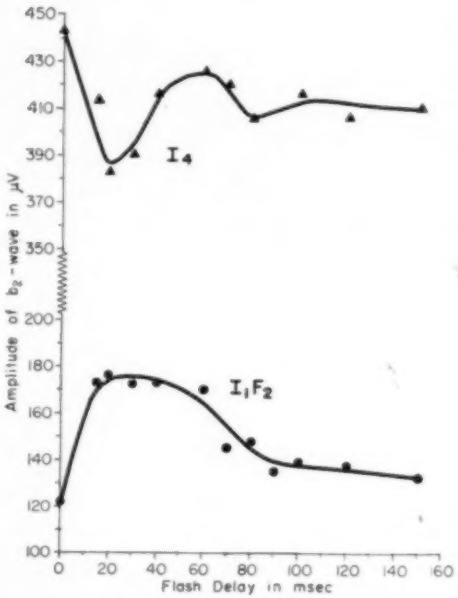

Fig. 4 (Burian and Spivey). Plot of average amplitudes of b_2-waves of first electroretinogram with single flash and with double flashes of increasing flash delay at intensities I_1F_2 and I_4. Note that for double flashes with short flash delays there is an increase in effective light intensity causing flashes at intensity I_1F_2 to increase the amplitude of the b_2-wave, whereas such flashes at intensity I_1 reduce the amplitude of the b_2-wave.

TABLE 2

AVERAGE AMPLITUDES IN μV OF COMPONENTS OF FIRST ELECTRORETINOGRAM AND OF B-WAVE OF SECOND ELECTRORETINOGRAM FOR STIMULUS INTENSITY I_1F_2

	First Electroretinogram			Second Electroretinogram
	Single flash	33 ± 3	122 ± 14	
Flash delay in msec. 15		40 ± 4	173 ± 21	—
20		33 ± 4	176 ± 19	—
30		37 ± 4	172 ± 17	—
40		30 ± 6	173 ± 15	—
60		40 ± 9	170 ± 24	—
70		31 ± 5	145 ± 19	—
80		33 ± 6	148 ± 17	—
90		30 ± 0	135 ± 6	—
100		37 ± 7	139 ± 11	27 ± 6
120		33 ± 7	137 ± 22	47 ± 9
150		33 ± 10	135 ± 23	83 ± 10

longer intervals were needed to obtain a second electroretinogram with the two lower intensity levels. However, even when no second electroretinogram appeared, twin stimuli had a definite effect on the first electroretinogram (fig. 1, tables 2 to 5).

At I_1F_2 the electroretinograms produced by 15 to 50 msec. delay interval flashes showed a considerable increase in the amplitude of the b_2-wave (maximal over 50 percent, fig. 4); the b_1-wave was not affected.

At I_1 there was an increase in the b_1-wave with 15 and 20 msec. flashes and a decrease in the b_2-wave; at I_4 and I_{16} there was under these circumstances a decrease of both the b_1 and b_2-waves.

As the interval between the flashes was lengthened, the b_2-wave tended to return almost fully to its original amplitude with I_1F_2 stimuli (fig. 4); with the three higher intensities it fell at first and then rose slowly, but never reached its original height, possibly because of the light adapting properties of the stronger stimuli (fig. 4). The b_1-wave fell at I_4 and I_{16} with flashes of 15, 20, and 30 msec. delay intervals and then remained throughout on the same level.

One may conclude from these findings that twin flashes with short delay intervals simply act by effectively increasing the stimulating light intensity. Thus such twin flashes produce a larger b_2-wave with low intensities (but are not strong enough to affect the b_1-wave or to cause the appearance of an a-wave); they inverse the relationship of the first and second positive components for

TABLE 3

AVERAGE AMPLITUDES IN μV OF COMPONENTS OF FIRST ELECTRORETINOGRAM AND OF B-WAVE OF SECOND ELECTRORETINOGRAM FOR STIMULUS INTENSITY I_1

	First Electroretinogram				Second Electroretinogram
	a_1	a_2	b_1	b_2	
Single Flash	97 ± 19	107 ± 16	373 ± 24	403 ± 16	—
Flash delay in msec. 15	97 ± 13	120 ± 11	403 ± 11	383 ± 20	—
20	83 ± 14	107 ± 10	392 ± 22	370 ± 24	—
30	87 ± 12	90 ± 10	370 ± 10	370 ± 25	—
40	90 ± 11	100 ± 10	363 ± 17	376 ± 8	—
60	83 ± 8	107 ± 6	390 ± 23	386 ± 16	—
70	87 ± 15	100 ± 9	393 ± 19	399 ± 20	13 ± 13
80	90 ± 14	103 ± 8	393 ± 18	393 ± 12	23 ± 18
100	87 ± 22	103 ± 13	376 ± 23	380 ± 27	43 ± 28
120	87 ± 9	97 ± 10	396 ± 25	380 ± 27	73 ± 24
150	87 ± 8	100 ± 9	386 ± 24	383 ± 21	103 ± 14

TABLE 4

AVERAGE AMPLITUDES IN μV OF COMPONENTS OF FIRST ELECTRORETINOGRAM AND OF B-WAVE OF SECOND ELECTRORETINOGRAM FOR STIMULUS INTENSITY I_4

		First Electroretinogram				Second Electroretinogram
		a_1	a_2	b_1	b_2	
Single Flash		230 ± 18	233 ± 86	479 ± 24	443 ± 13	—
Flash delay in msec.	15	223 ± 13	240 ± 6	459 ± 27	413 ± 42	—
	20	213 ± 10	230 ± 6	446 ± 24	383 ± 50	—
	30	206 ± 15	214 ± 8	436 ± 28	390 ± 60	—
	40	213 ± 15	220 ± 5	443 ± 30	416 ± 32	23 ± 10
	60	206 ± 10	206 ± 5	446 ± 30	426 ± 53	31 ± 17
	70	213 ± 14	216 ± 10	446 ± 33	420 ± 53	50 ± 30
	80	203 ± 18	213 ± 11	446 ± 26	406 ± 59	63 ± 31
	100	209 ± 12	216 ± 13	450 ± 30	416 ± 64	93 ± 41
	120	200 ± 20	210 ± 18	446 ± 33	406 ± 68	133 ± 43
	150	216 ± 5	220 ± 7	450 ± 24	410 ± 53	170 ± 43

intermediate intensities, and create supramaximal stimuli for high intensities. These results may again be interpreted in terms of a differentiation of the photopic and scotopic components of the electroretinogram.

An intensity effect of twin flashes is also evident from the series of electroretinograms for the "dark-adapted" eyes reproduced by Mahneke,[2] although he draws no attention to this fact. Judging from his reproductions, the b-wave measured for very short flash intervals roughly 500 μV, whereas for flash intervals of over 100 msec. it measured about 330 μV.

3. THE SECOND ELECTRORETINOGRAM

The appearance of a second electroretinogram with twin flashes in relation to the duration of the delay interval depends upon the intensity of the flashes: the *weaker* the intensity, the *longer* must be the interval (tables 2 to 5 and fig. 5).

At intensity I_1F_2 a second electroretinogram was recorded in all records with a 100 msec. flash delay; in two records there was evidence of a second electroretinogram with a 70 msec. flash delay and in four with a 80 msec. flash delay.

At intensity I_1 a second electroretinogram was recorded in all records with a 70 msec. flash delay; in four records there was evidence of a second electroretinogram with a 60 msec. flash delay.

At intensity I_4 a second electroretinogram

TABLE 5

AVERAGE AMPLITUDES IN μV OF COMPONENTS OF FIRST ELECTRORETINOGRAM AND B-WAVE OF SECOND ELECTRORETINOGRAM FOR STIMULUS INTENSITY I_{16}

		First Electroretinogram				Second Electroretinogram
		a_1	a_2	b_1	b_2	
Single Flash		276 ± 17	262 ± 29	456 ± 47	433 ± 63	—
Flash delay in msec.	15	266 ± 25	240 ± 43	410 ± 67	406 ± 57	—
	20	280 ± 16	273 ± 18	423 ± 60	403 ± 43	—
	30	270 ± 17	260 ± 32	426 ± 50	396 ± 70	—
	40	276 ± 21	263 ± 27	409 ± 80	406 ± 80	24 ± 4
	60	266 ± 16	253 ± 22	400 ± 70	403 ± 47	50 ± 32
	70	256 ± 28	250 ± 32	393 ± 80	400 ± 80	47 ± 28
	80	270 ± 22	250 ± 25	403 ± 83	403 ± 57	63 ± 34
	100	263 ± 19	253 ± 22	393 ± 83	390 ± 60	90 ± 32
	120	273 ± 24	260 ± 29	396 ± 84	396 ± 50	130 ± 24
	150	273 ± 21	260 ± 31	396 ± 83	403 ± 80	160 ± 22

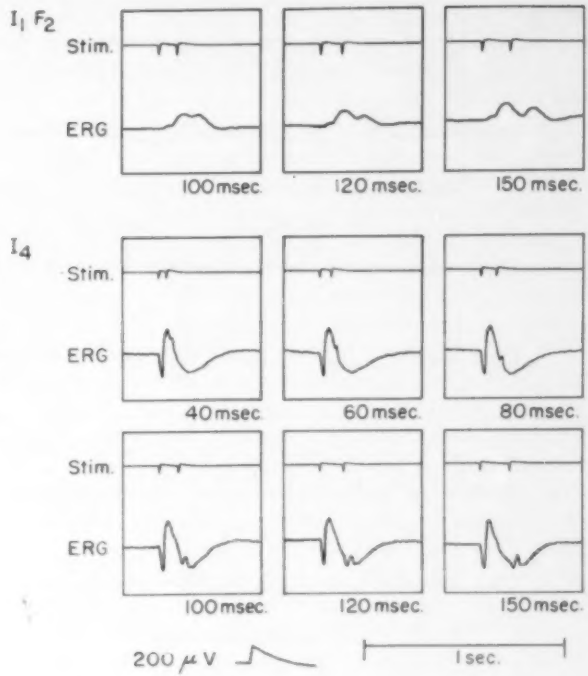

Fig. 5 (Burian and Spivey). Second electroretinogram produced with twin flashes at intensity I_1F_2 (upper row) and I_4 (second and third row). Note that the shapes of the second electroretinograms remain essentially unchanged at I_1F_2 whereas their shape differs greatly from the first electroretinograms at I_4.

was recorded in all records with a 40 msec. flash delay; in three records there was evidence of a second electroretinogram with a 30 msec. flash delay.

At intensity I_{16} a second electroretinogram was recorded in all records with a 40 msec. flash delay; in four records there was evidence of a second electroretinogram with a 30 msec. flash delay.

The character of the second electroretinogram was always essentially photopic, consisting of a single positive elevation with a sharp rise for the higher intensities but for flash delays of 120 and 150 msec. a very small positive elevation could be seen following the first sharp rise, representing most likely the first indication of a b_2-wave. The amplitude of the second electroretinogram increased with prolonged flash delay, except for intensity I_1F_2 where the amplitude remained at the same low level for the relatively short flash delays of 100, 120, and 150 msec. (fig. 6).

Why must the flash delay be greater for low intensities than for high ones, in order that the second flash may give rise to an

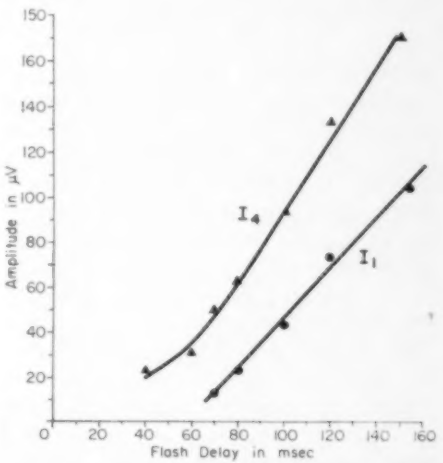

Fig. 6 (Burian and Spivey). Average amplitude of b-wave of second electroretinogram with stimuli of intensities I_1 and I_4.

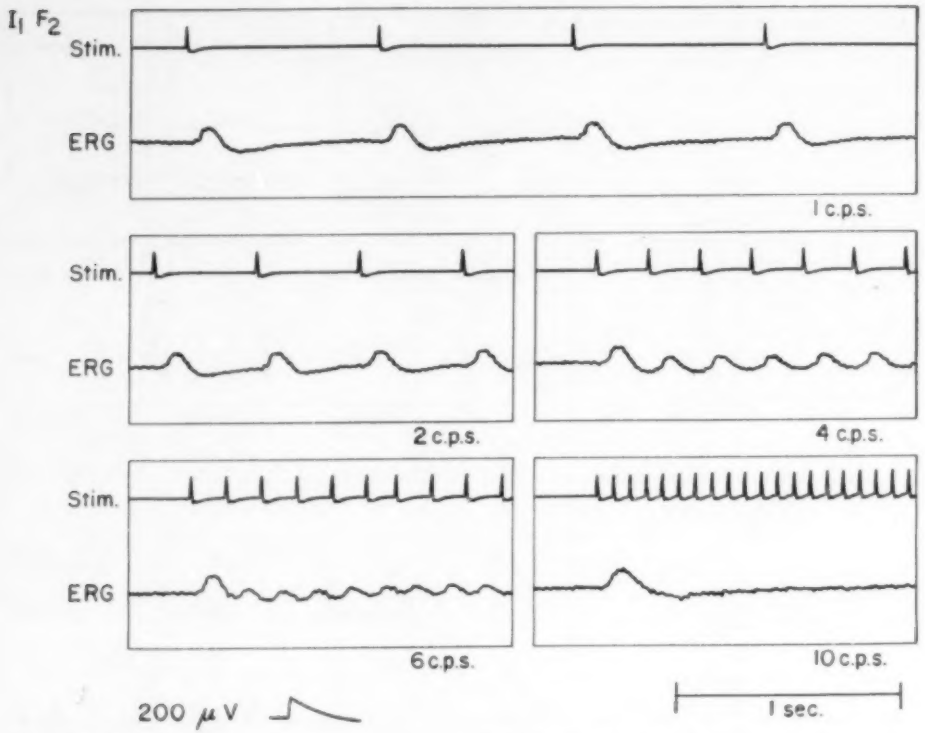

Fig. 7 (Burian and Spivey). Electroretinograms obtained with repetitive light stimuli at intensity I_1F_2. Note that there is no change in shape, but only a reduction in size, with increasing stimulus frequency. Note also the larger size of the one and only electroretinogram at frequency 10 cps which in this case represented the fusion frequency.

electroretinogram response? Low light intensities stimulate presumably the slower scotopic mechanism, whereas high light intensities stimulate the faster photopic mechanism. It is known from the study of electroretinograms produced with repetitive flashes that the scotopic mechanism cannot follow fast stimulus frequencies and that the fusion frequency is very much lower for low intensity flashes than it is for high ones. The requirement of a larger flash delay for low intensities, to elicit twin electroretinograms, would seem to be readily explained on this basis. Nevertheless, this greater flash delay requirement raises certain questions which will be discussed in the following section dealing with the electroretinograms obtained with repetitive light stimuli.

4. The electroretinogram with repetitive flashes

Electroretinograms were obtained with repetitive stimuli ranging in frequency from one stimulus per second to 20 stimuli per second, that is, with intervals between stimuli from 1,000 to 50 msec.

We shall in this presentation pay particular attention to the electroretinogram following the first stimulus and to the shape of the subsequent stimuli.

For all recordings, regardless of the stimulus frequency, the first electroretinogram

was always of considerably larger amplitude in all its component waves than were the subsequent electroretinograms. This was particularly evident for the second electroretinogram and increasingly so for higher stimulus frequencies. The second electroretinogram not only was lower in amplitude, but had a definitely "photopic" character for the higher intensities. The subsequent electroretinograms depended for their shape and amplitude on the intensity of the stimulus and on its frequency.

At I_1F_2 (fig. 7) there was, with a stimulus frequency of one and two cps, essentially no difference in either amplitude or shape between the first electroretinogram and the subsequent ones. From the beginning a steady response was obtained. With a frequency of four cps the second electroretinogram was notably smaller than the first, and all subsequent electroretinograms were equal to the second. No difference in shape between first and subsequent electroretinograms could be noted. With increasing frequency the electroretinograms which followed the first one became progressively smaller and fusion was soon obtained—with eight or 10 cps. This is consistent with the low flicker fusion frequency of the scotopic mechanism. It should also be noted in Figure 7 that the amplitude of the one and only electroretinogram obtained with 10 cps, elicited in response to two stimuli, is considerably larger than the amplitude of the electroretinogram obtained with lower frequencies.

The electroretinograms elicited by repetitive stimuli of I_1 I_4, and I_{16} are much more complex than the ones elicited with I_1F_2. With all the higher intensities the second electroretinogram became the smaller, the faster the flicker, and for the frequencies corresponding to those employed in producing twin flashes, the second electroretinograms encroached upon the first electroretinogram and were quite comparable to those described in the preceding section of this paper. However, after three, or at the most five, flashes the electroretinograms attained a size and shape characteristic for the particular intensity and particular frequency. It is essential to note that this steady response could be maintained indefinitely.

As has been mentioned before, this steady response was indistinguishable from the first electroretinogram for frequencies of one and two cps with I_1F_2; for higher frequencies it differed only in the size of the electroretinogram. The steady responses with I_1 still had some "scotopic" characteristics: a small a-wave and a rounded b-wave (fig. 8). With I_4 the a-wave was comparatively larger; the b-wave became a sharp, narrow peak. At lower frequencies of repetitive stimuli a double b-wave could still be made out, but the second peak quickly disappeared with increase in frequency (fig. 9). Finally, with I_{16} the a-wave was larger still, the b-wave further reduced in height, and a trend toward a positive elevation preceding the a-wave could be made out (fig. 8).

The existence of a refractory period which determines the delay in the appearance of the second electroretinogram with twin flashes, and the observations made with repetitive stimuli, require an explanation.

Granit[12] concluded on the basis of his beautiful experiments on single fibers of the optic nerve of animals that the refractory period and the reduction in the b-wave were due to postexcitatory synaptic inhibition, whereas the appearance of larger a-waves with multiple stimulation resulted from a pre-excitatory inhibition of the off-effect. Dodt[1] has taken over these concepts of Granit's and enlarged them by applying them to human electroretinography. In particular, he has shown that by suppressing the scotopic activity of the human retina by means of flicker the photopic activity can be brought out, including the presence of an off-effect.

Adrian[13] offered a different explanation. Calling attention to the reduction in the size of the scotopic component after the first response, observed in flicker experiments, he

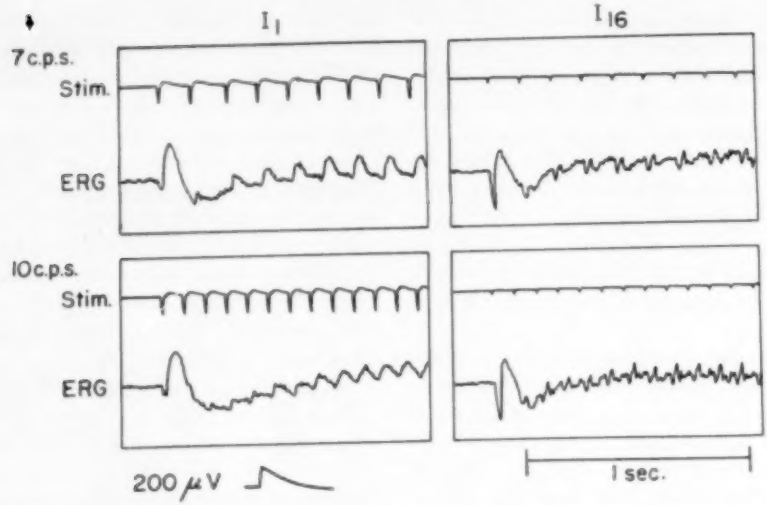

Fig. 8 (Burian and Spivey). Electroretinograms obtained with repetitive light stimuli at intensities I_1 (left column) and I_{16} (right column). Note in the I_1 records the relatively small a-wave and the relatively large b-wave which is rounded in shape (prevalence of b_2-wave). At I_{16} the a-wave is larger, the b-wave smaller, more peaked in shape (prevalence of b_1-wave).

stated that it was presumably due to the light-adapting effect of the first flash. This finding suggested to Adrian a dependence on photochemical factors. He added, however, that the rate of recovery might well depend on neural factors also. Adrian found furthermore that with the use of blue light the responses did not become progressively smaller, but that after the first few flashes the responses attained a steady value "as though the retina discharged its accumulated reserves at the first stimulus, but could then revert to a state of dynamic equilibrium where the supply keeps pace with the demand."

This view has much to attract the ophthalmologist, fitting, as it does, so well with what we know about the origin of the retinal potentials and with many observations in clinical electroretinography. Experimentally, a close correlation of photochemical processes and size and shape of the electroretinogram has been demonstrated in the recent work of Dowling and Wald[14] who showed that there exists a remarkable parallelism between the electroretinographic threshold and the rhodopsin content in the retinas of vitamin A-deficient rats.

One feels justified, therefore, to explain the observations with twin flashes of light and with repetitive light stimuli by the content in photopigments of the retinal sensory elements.

The amount of photopigments in the sensory retinal elements available under specified conditions would seem to be fully capable of accounting for the observations made with double light flashes and repetitive light stimuli. The delay required for the appearance of the second electroretinogram, the reduction in size and change in shape of the second electroretinogram are readily explained by it. The attainment, with repetitive stimuli, of the steady dynamic equilibrium spoken of by Adrian,[13] characterized by electroretinograms typical for each level, having larger a-waves and smaller b-waves with progressively higher intensities and greater frequencies, causing an ever more light adapted state of the eye, further indicate that

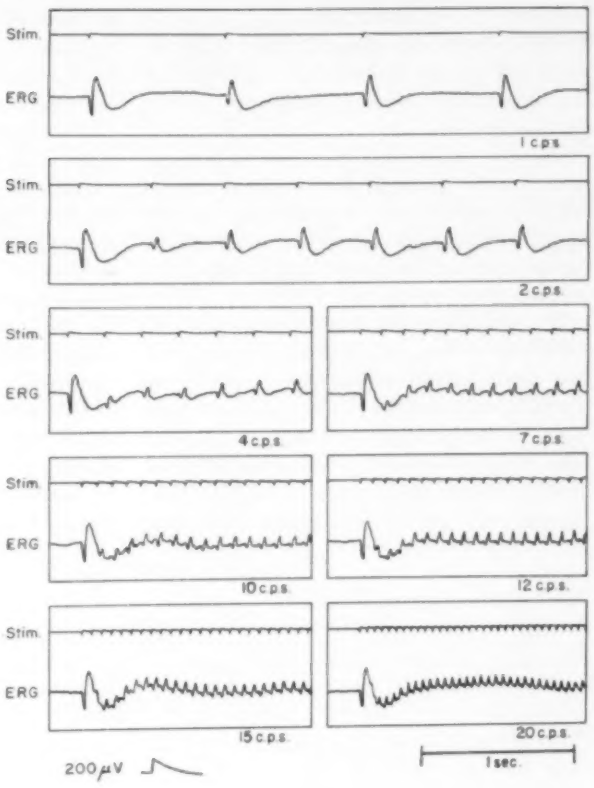

Fig. 9 (Burian and Spivey). Electroretinograms obtained with repetitive stimuli at intensity I_4. Note that a constant response is established in each record after one (one cps) to six or seven (15 and 20 cps) stimuli following the first stimulus. Note, furthermore, the almost immediate reduction in the b_2-wave, as well as the steady reduction in size of the a- and b_1-waves, with progressively higher frequencies of stimulation. Note, lastly, that the second electroretinogram with seven, 10, 12, 15, 20 cps agrees well with the electroretinograms obtained with the double flashes of the roughly corresponding flash delays of 150, 100, 80, 70, 50 msec.

the size and shape of the electroretinogram depend on the level of available photopigments, resulting from their bleaching and resynthesis.

In considering the results of our study, it must be kept in mind that it was performed with stimuli of extremely short duration (10 microseconds, according to the manufacturer) and very high intensity. This may well explain discrepancies between our findings and those of other observers. Dodt[1] found it necessary, for instance, to reduce the scotopic activity by flicker, in order to obtain maximal a-waves of 100 μ V. A-waves of comparable size were never observed by him either with single or double flashes. With our photo stimulator a-waves of 100 μ V are commonplace and with higher intensities we obtain frequently a-waves of 200 to 250 μ V in normal eyes. Also, Dodt stated that the optimal frequency to elicit the maximal a-wave was eight to 10 cps. Greater or smaller frequencies would give a-waves of smaller size. Dodt has explained this observation by the fact that the optimal condition for the production of an a-wave is given when the second stimulus arrives at the peak of the off-effect, basing himself on Granit's view that the a-wave is an expression of the pre-excitatory inhibition of the off-effect. From our results it would appear that the size of the a-wave with repetitive stimuli may very well also be simply a function of stimulus intensity. A comparison of the a-waves obtained with light flashes of I_1, I_4, and I_{16} (figs. 8 and 9) would certainly point in this direction.

SUMMARY

This report is concerned with a study of twin light flashes and repetitive light stimuli on the human electroretinogram of five young subjects.

An analysis of the electroretinogram obtained with the single flashes allows one to attribute once again the first and second positive elevations to the photopic mechanism, the second negative and positive elevations to the scotopic mechanism.

To elicit a second electroretinogram with twin flashes longer flash delays are required for low intensities than for higher ones. Even if no second electroretinogram is produced by twin flashes with short delay intervals, the second flash does have an effect on the first electroretinogram: at low flash intensities the second flash caused the total intensity to be effectively increased; at intermediate flash intensities the quantitative relationship of the first and second positive and negative components of the electroretinogram was reversed; at high intensities supramaximal stimuli were created.

After the first few flashes, repetitive stimuli give rise to a steady response which is characteristic for each level of intensity and frequency. In general, the electroretinograms obtained with higher stimulus intensities and greater stimulus frequencies are more complex and tend to assume a more and more "photopic" character.

The authors favor an explanation of the observed phenomena based on the dependence of the electroretinogram on photochemical processes.

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AUTONOMIC INNERVATION OF THE CILIARY BODY*

A MODIFIED THEORY OF ACCOMMODATION

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One of the more interesting and controversial aspects of ocular physiology in recent years has been the relative importance of parasympathetic and sympathetic portions of the autonomic nervous system on visual accommodation. As early as 1801 Young[1] proposed that changes in the refractive power of the eye were brought about by changes in the radius of curvature of the crystalline lens, and not by changes in eyeball axial length, corneal curvature or position of the lens. The Young hypothesis was rediscovered by Helmholtz[2] who strongly championed this mechanism of refractive change. The "Helmholtz theory of accommodation," which is almost universally accepted, today, states that when the eye accommodates for a near object, the ciliary muscle contracts, opposing the elastic force of the choroid layer, decreasing the tension exerted by the suspensory ligament on the lens capsule thereby allowing the elastic forces of the capsule to mold the lens to a more spherical form.[3] This action of the ciliary muscle results when it is stimulated by the oculomotor nerve, part of the parasympathetic system.

The role and relative importance of the sympathetic system in controlling accommodation has been less clearly delineated. Since Horner's[4] initial observation in 1869, it has been known that individuals with long standing cervical sympathetic lesions evidenced no apparent alteration of distance accommodation. This phenomenon is in contrast with the findings of Morat and Doyon[5] who reported that stimulation of the cervical sympathetic nerve will result in a decrease in refractive power of the eye as well as pupillary dilation. These results have been verified many times over by Cogan[6] and Morgan and Olmsted.[7] The latter authors observed that an excited rabbit becomes hyperopic. Repeating this experiment on human subjects they found that most individuals become hypermetropic when subjected to a sudden startling stimulus.[8]

This flattening of the crystalline lens occurs as part of the generalized sympathetic response, dilated pupils, elevation of blood pressure, decreased skin resistance and an increase of heart rate. Morgan, Olmsted and Watrous[9] verified the observations that cervical sympathetic stimulation decreased the refractive power of the eye in cats, dogs and monkeys. In addition, sectioning the oculomotor nerve in the cat augmented this response. Olmsted and Morgan stated that similar results were obtained when photographs of the Purkinje-Sanson images were used to determine changes in refractive power resulting from sympathetic stimulation.[10] Fleming and Olmsted[11] determined the immediate effects of superior cervical ganglionectomy on the refractive power of the eye in cats and rabbits. They reported that there is an immediate increase in refractive power in the eye on the side of the lesion, but that this difference becomes insignificant after several days. In addition the sympathetically innervated portion of the accommodative mechanism develops a supersensitivity response to intravenously administered epinephrine five days after denervation.

The experimental results regarding the

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effects of sympathetic stimulation are quite well documented, however, there is considerable dispute regarding the actual mechanism involved.

Henderson[12] first proposed that the ciliary muscle was innervated by both sympathetic and parasympathetic nerve fibers, but had little experimental evidence on which to base his conclusions. Kuntz, Richins, and Casey[13] advanced a theory which maintained that the action of the sympathetic system on accommodation was mainly inhibitory. They believed that all the nerve fibers acting directly to alter the refractive power of the lens came from the parasympathetic system via the oculomotor nerve. They concluded that the third cranial nerve contained parasympathetic fibers with adrenergic endings and that the cervical sympathetic nerve acted on the mid-brain to inhibit the oculomotor nerve.

This hypothesis was weakened by the experimental results of Melton, Parnell and Brecher[14] who stimulated the long and short ciliary nerve fibers in enucleated, perfused cats eyes. These investigators observed different patterns of ciliary muscle contraction when the long and short ciliary fibers were stimulated. They concluded that in all likelihood the ciliary muscle had direct, dual innervation. In an attempt to resolve the enigma that stimulation of the cervical sympathetic nerve brought about negative accommodation, while ablation of the same nerve produced only a transient increase in refractive power the following series of experiments were undertaken.

Materials and methods

An experimental procedure was devised to investigate the gross and microscopic changes which take place within the eye as the result of prolonged unilateral sympathetic stimulation with and without parasympathetic ablation. Twenty-nine cats were employed as the experimental animals.

The exposed cervical sympathetic nerve on one side in nembutalized cats was stimulated with rectangular pulses of 10 milliseconds' duration at a rate of 20 per second and of sufficient intensity to maintain maximal dilitation for a three hour period. At the end of this interval and while the stimulus was still being applied, both eyes were fixed in vivo by infusing a four-percent aqueous lead subacetate solution through the corneas into the anterior chambers. This resulted in an almost immediate fixation of the anterior segments bilaterally. The eyes were enucleated and immersed in the fixative for 24 hours.

The eyes were washed and infiltrated with dilute gelatin solutions from two to 10 percent, blocked, and hardened with McManus fluid under refrigeration and sectioned at 20 micra on a sliding microtome.

Sections were stained with the toluidine blue method of Richins and Hall.[15] In a series of six animals the ciliary ganglion was extirpated from the right eye. After allowing adequate time for recovery, the cervical sympathetic trunk was stimulated on the contralateral side as described above. The eyes were removed after in vivo fixation with four-percent aqueous lead subacetate solution.

Lead subacetate fixation, is a further modification of Hess and Hollanders[16] toluidine blue method for metachromasia. Benzene was substituted for acetone by Richins and Hall. Lead subacetate proved to be an excellent fixative for this study since pupillary action could be instantaneously "fixed." It is essential for the best results, however, that this solution be made CO_2 free by boiling before use.

In a parallel procedure the superior cervical sympathetic ganglion was extirpated unilaterally in six cats. The animals were allowed to recover and the contralateral cervical sympathetic trunk was electrically stimulated. The eyes were fixed in vivo, removed and treated as the other tissues.

The oculomotor nerve was cut intracranially on one side in three animals and after time for recovery the cervical sympathetic

trunk on the contralateral side was exposed and stimulated. The standard procedures described above were followed.

In animals used as sham stimulated controls one from each of the surgical groups produced by the above procedures was selected. After adequate time for recovery from surgery which was usually three to four weeks the cervical sympathetic trunk was exposed on the contralateral side under nembutal. The electrodes were placed in contact with the trunk but the stimulus was not applied. The animals were kept in this position for three hours simulating the experimental techniques. The eyes were fixed in vivo and handled in the same manner as the experimental tissues. Sections from the two eyes were mounted on the same slide and stained simultaneously.

Results

Superior cervical ganglionectomy

In 11 nembutalized cats just the exposed cervical sympathetic nerve was stimulated. The ipsilateral pupil dilated maximally while the pupil in the contralateral eye constricted maximally (fig. 1). This represents a contra-consensual pupillary response which could not be overridden or modified by varying the level of light entering either or both eyes. As described in the method above both eyes were perfused within minutes of each other and retained their relative pupillary diameters. It was possible to observe a dilated pupil in the fixed, enucleated eye on the stimulated side in a constricted pupil and its mate.

Saggital sections of stained eyes using the toluidine blue method revealed a flattened lens on the stimulated side, and a rounded spherical lens on the contralateral unstimulated side. The ciliary body in each of the two eyes demonstrated a marked degree of activity, as indicated by a color change from blue to green of the ciliary muscles. This change in color apparently indicates altered metabolic activity elicited by stimulation. These changes in staining characteristics were demonstrated in autonomic ganglia following preganglionic stimulation by Richins and Hall.[15] Their ability to prevent and control this response in neurones of the superior cervical ganglion by using various drugs established this method as a research tool.

Our findings in the present investigation seemed to justify further use of this method. The ciliary body in both eyes showed the same relative degree of activity, although the cervical sympathic nerve was stimulated on one side. Localization of activity within the ciliary muscle was not possible. Blood vessels within the ciliary body in the stimulated eye showed a marked degree of constriction while those in the contralateral eye appeared dilated.

Ciliary ganglionectomy—Cervical sympathetic stimulation

In those eyes with the ciliary ganglion removed a dilated pupil and a flattened lens were observed (fig. 2). In the contralateral eye which was stimulated sympathetically similar responses were demonstrated but to a greater degree. Histological examination of the stained anterior segments of both eyes revealed marked activity on the stimulated side with little or no activity in the eye which had been previously parasympathetically denervated.

Fig. 1 (Fleming and Hall). Photograph taken while the cervical sympathetic trunk on the right side is stimulated. Note dilitation of right pupil and constriction of the left. Nictitating membrane of left eye is retracted with a ligature.

Fig. 2 (Fleming and Hall). Effects of extirpation of the right ciliary ganglion. Cat is still under the effects of surgical anesthesia. Note dilitation of right pupil.

Fig. 3 (Fleming and Hall). Cat with unilateral extirpated superior cervical ganglion. Note the Horner's syndrome on the right side which was of two years' duration in this animal.

Superior cervical ganglionectomy—Cervical sympathetic stimulation

In a subsequent procedure the superior cervical ganglion was removed on one side, and the animals were allowed to recover for variable periods of time ranging from several days to two years (fig. 3). The cervical sympathetic nerve on the contralateral side was stimulated. Sections of the eyes showed marked differences. The lens was flattened in the eye on the stimulated side while on the unstimulated side the lens had a smaller radius of curvature. The vessels in the ciliary body on the side stimulated showed marked constriction as compared to those in the body of opposite eye. The ciliary bodies of both eyes showed approximately the same relative degree of metabolic activity.

Third nerve lesion—Cervical sympathetic stimulation

The animals that had the third nerve severed intracranially presented the same histological picture as those with the ciliary ganglionectomy. These results were expected since they represented the same essential procedure except for the fact that there was no trauma within the orbit itself.

Controls

One cat from each of the experimental surgical groups and a normal animal were subjected to sham stimulation for three hours of the cervical sympathetic trunk on the intact side. Color changes observed deviated from the controls only in the eyes on the denervated side. The ciliary body of the normal animal showed slight blue to green color changes.

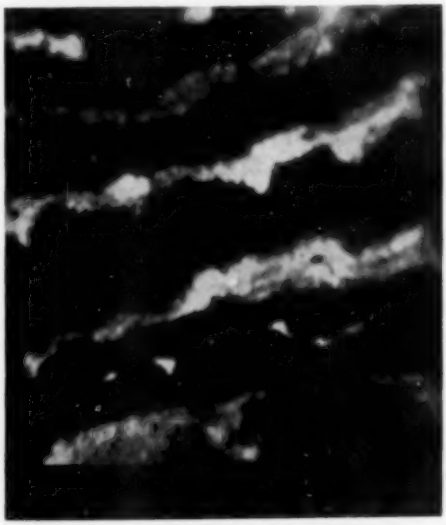

Fig. 4a (Fleming and Hall). Photograph through blood vessels of the ciliary body. Appearance of blood vessels after three-hour stimulation of the cervical sympathetic nerve on the same side. Note the markedly constricted blood vessels. An intense color-change was observed. (Magnification ×1250.)

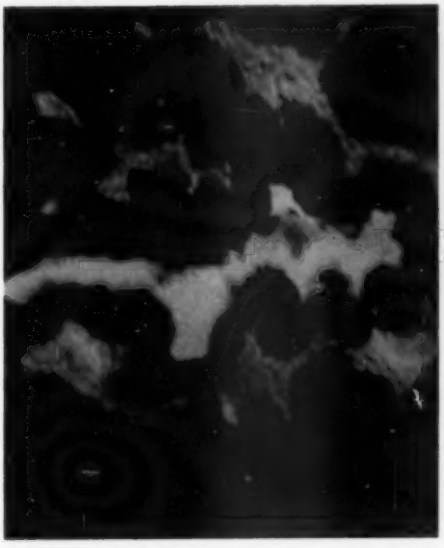

Fig. 4b (Fleming and Hall). Photograph through blood vessels of the ciliary body. Appearance of blood vessels of the body after three-hour stimulation of the cervical sympathetic nerve on the opposite side. A good blue-green color change was present but the vessels' diameters were of the same order of caliber as the controls. (Magnification ×1250.)

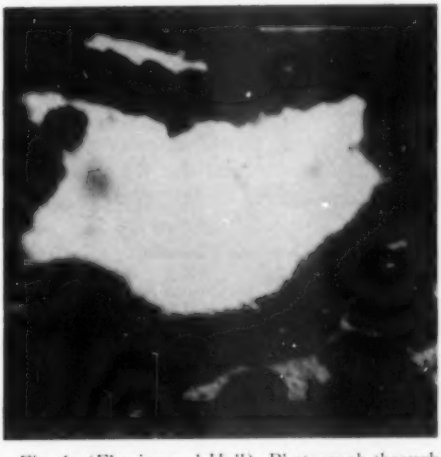

Fig. 4c (Fleming and Hall). Photograph through blood vessels of the ciliary body. Dilated arteriole 24 hours after extirpation of the superior cervical ganglion ipsilaterally. The cervical sympathetic nerve was stimulated contralaterally for three hours. A blue to green color change was still present. (Magnification ×1250.)

Fig. 4d (Fleming and Hall). Photograph through blood vessels of the ciliary body. Blood vessels of the ciliary body in an eye with parasympathetic denervation (ciliary ganglionectomy). Cervical sympathetic nerve stimulated on the contralateral side for three hours. Blood vessels were of the same order of magnitude as controls. There was a complete absence of color change. (Magnification ×670.)

Discussion

Prolonged stimulation of the cervical sympathetic nerve on one side elicited color changes from blue to green in the ciliary body of both eyes. Constricted blood vessels in the body, however, were seen only in the eye stimulated. To test for the possibility of reflex parasympathetic stimulation of the ciliary muscle in the eye on the contralateral side through a central mechanism, the ciliary ganglion was extirpated unilaterally in six cats and the oculomotor nerve was sectioned unilaterally in three additional animals. In both of these groups with parasympathetic lesions the color changes in the denervated eyes were markedly inhibited as compared to the non-lesion animals.

Eyes removed from a control anesthetized animal subjected to no prior surgical trauma exhibited basal metabolic activity as determined on the basis of this staining technique.

A modified theory of accommodation

As stated in the introduction, the problem of sympathetic action on accommodation

was still to be resolved. A theory of sympathetic action on accommodation should fit the following data:

a. The system responds to direct sympathetic stimulation with an increase in negative accommodation.

b. Humans and experimental animals with long-standing lesions of the cervical sympathetic system as seen in Horner's syndrome, do not manifest alterations of distance accommodation in either eye. Cogan,[17] however, has reported that patients with cervical sympathetic lesions exhibit a greater accommodative amplitude in the eye on the side with the lesion. In addition, Morgan[3] states that the administration of amphetamine sulfate will cause a reduction of accommodative amplitude.

c. Stimulation of long and short ciliary fibers produces a difference in the pattern of ciliary muscle contraction while ablation of the superior cervical ganglion causes only a transient increase in refractive power.

d. The histophysiologic data reported in the present paper led to the conclusion that sympathetic stimulation caused constriction of the blood vessels in the ciliary body while ablation of the same nerve will cause a rapid dilation of the blood vessels in the ciliary body. In addition there was some evidence that different segments of the muscle responded to sympathetic and parasympathetic stimulation.

On the basis of the foregoing body of information the following hypothesis was developed:

The primary mechanism for the control of accommodation is through the action of the oculomotor nerve on the ciliary muscle as conceived in the original Young-Helmholtz theory. In addition the sympathetics alter accommodation in one and perhaps two ways. Most of the sympathetic nerve endings in the ciliary body terminate in the walls of blood vessels. When the sympathetics are stimulated the blood vessels constrict decreasing the blood volume of the ciliary body. Since the body is firmly fixed to the sclera at its outer margin, a decrease in body size results in an increase in the tension exerted by the suspensory ligament on the lens capsule and an ultimate decrease in the refractive power of the lens. Conversely when the cervical sympathetic system is interrupted there is an immediate decrease in vasomotor tone and an increase in ciliary body blood volume. This reduces the tension exerted by the zonule and an increase in the refractive power of the lens. After several days there is the return of the inherent muscle tone in these blood vessels and this is common to denervated vascular structures in many parts of the body.

The selective color changes in the ciliary muscle produced by direct stimulation of the cervical sympathetic nerve as well as the data of Melton, Purnell and Brecher indicates that there might be an additional direct action of the sympathetic system on parts of the ciliary muscle, and that the muscle possesses some degree of dual innervation.

Lamp Division,
General Electric Company,
Nela Park.

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Discussion

Dr. Julia T. Apter, Chicago: Several aspects of this paper are indeed ingenious and commendable. The method of fixing the eyes of experimental animals while the iris is actually responding to some stimulus will be copied by many others engaged in similar work. I also am interested in the differential staining of stimulated and unstimulated areas in the ciliary body. However, I would like to point out some drawbacks in any method that attempts to demonstrate function of a nerve pathway by electrical stimulation of that pathway.

When a ganglion is stimulated by an electric current, several types of events are set off: (1) A shock artefact travels both centrifugally and centripetally in all fibers connected to the ganglion without regard to whether these fibers are normally afferent or efferent; (2) an action potential similar to a normal action potential may follow the shock artefact but this will travel down the nerves in the normal direction and it is capable of initiating a normal response in the end organ. There is good reason to believe that such a normal action potential is set off by only specific kinds of electric stimuli; (3) action potentials entirely different from the normal ones may be initiated and would then produce in the end organ responses of an abnormal nature. The shock artefact, too, would induce abnormal responses in the end organ since it would act simply like an electric shock to the end organ and not like a normal nerve impulse.

When electrical stimulation is used on nerves, the shock artefact is always present although it can be minimized with an isolation unit. Other errors inherent in electrical stimulation may be reduced if parameters of current are chosen that initiate action potentials indistinguishable from the normal action potential, that is, the potential usually preceding the observed response in the end organ. If the form of the action potential normally initiating the response is an unknown quantity, then we must be cautious, indeed, about interpreting the response induced by an arbitrarily chosen electric shock stimulus.

In this presentation I did not notice any mention of an isolation unit. What is more, I know that action potentials have never been observed in the sympathetic ganglia during changes in ciliary muscle activity in cats. Therefore, we must assume that some of the responses in these animals were due to direct electrical stimulation of the ciliary body and were not necessarily mediated through the sympathetic ganglion. Even the responses described here in the contralateral eye could be explained by retrograde transmission of the electric shock to the hypothalamus and thence down other autonomic pathways to the unstimulated side.

Previous work indicates that further work is certainly necessary to elucidate the control the orthosympathetic system may have over accommodation. Therefore I certainly would wish to encourage these workers who recognize this need. However, the limitations of the methods used here should be kept in mind before making interpretations of the data.

EVALUATION OF ANTERIOR AND POSTERIOR TRABECULODIALYSIS*

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In a previous report[1] two procedures, the anterior and posterior trabeculodialysis, were described by which the corneoscleral trabeculum can be detached from its bed leaving Schlemm's canal anatomically open.

In the present paper a comparison is made between the two methods and the attempt is undertaken to evaluate statistically the anatomical results obtained. For this study the serial meridional sections of 58 cases operated with anterior trabeculodialysis and of 63 cases operated with posterior trabeculodialysis were used.

COMPARISON OF OPERATIONS

Technically, both operations are easy to perform successfully, though in my experience posterior trabeculodialysis is generally a smoother procedure than anterior trabeculodialysis. The following points in particular should be mentioned here:

1. In anterior trabeculodialysis when performing the sweeping movements, the treated sections of the iris and the pupil are drawn by the blade of the angulated spatula toward the angle of the anterior chamber. Though the iris usually slips back spontaneously, gentle massage at the limbus with a blunt instrument such as a strabismus hook is sometimes necessary until it recovers its normal position.

2. In anterior trabeculodialysis during the sweeping movement of the blade the actual detachment of the corneoscleral trabeculum is felt by the fingers holding the handle of the spatula. The sensation is that of passing over a narrow furrow, that is, Schlemm's canal, with the edge of the blade. During the last (third) sweep in this procedure the tip of the blade sometimes becomes entangled in the scleral tissue, most probably at the posterior aspect of Schlemm's canal near the scleral spur and cannot be rotated further. If the movement is forced it is natural that the sclera will be injured but this can be avoided by retracting the blade 1 to 1.5 mm.; the tip of the blade becomes disentangled and the sweep can be then easily completed.

The situations described under paragraphs 1 and 2 do not occur in posterior trabeculodialysis.

3. In anterior trabeculodialysis owing to anatomic variations of the width of the limbus, the blade of the spatula has to be rotated and carried up to four mm. posterior to the limbus in order to assure that the corneoscleral trabeculum has been reached and treated by the active edge of the blade. Thus, it is inevitable that the separation of the ciliary body is wider and the possible postoperative irritation of this structure might be greater than in posterior trabeculodialysis.

4. As mentioned above, the blade of the spatula must be carried sufficiently back in to the subchoroidal space otherwise it is obvious that there would be no effect on the structures of the angle. In some sections of this series the corneoscleral trabeculum was found detached from its anterior insertion near Descemet's membrane while still attached to the scleral spur; the ciliary body also remained attached to the scleral spur so that no subchoroidal cleft was formed (figs. 1 and 2). This incomplete anterior trabeculodialysis would theoretically be the ideal trabeculodialysis since the aqueous can reach the outer aspect of Schlemm's canal and no coincidental cyclodialysis occurs. It is, however, most probable that in such a case the corneoscleral trabeculum would reattach to its bed due to the postoperative irritation however slight this might be. Besides,

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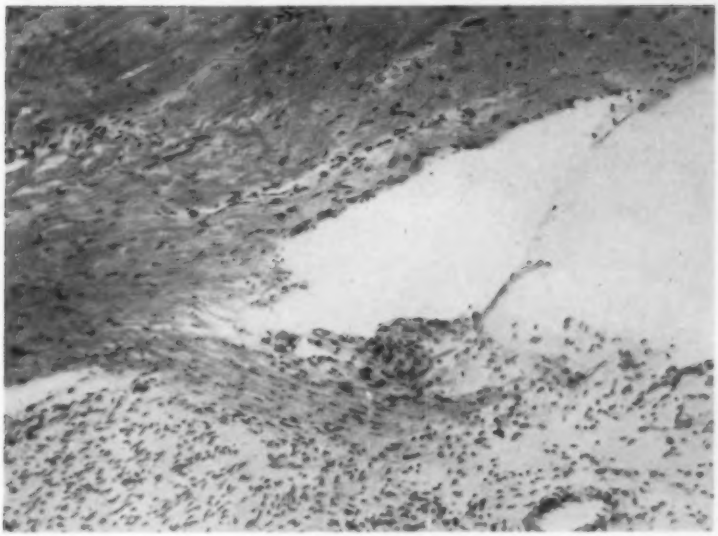


Fig. 1 (Dellaporta). Incomplete anterior trabeculodialysis where ciliary body and base of corneoscleral trabeculum remained attached to the scleral spur but Schlemm's canal is anatomically open. Arrow indicates the previous anterior attachment of the corneoscleral trabeculum. In this case the aqueous humor has free access to the outer aspect of Schlemm's canal. Well visible endothelium of Schlemm's canal. Section derives from random segment of an eye quadrant treated by anterior trabeculodialysis.

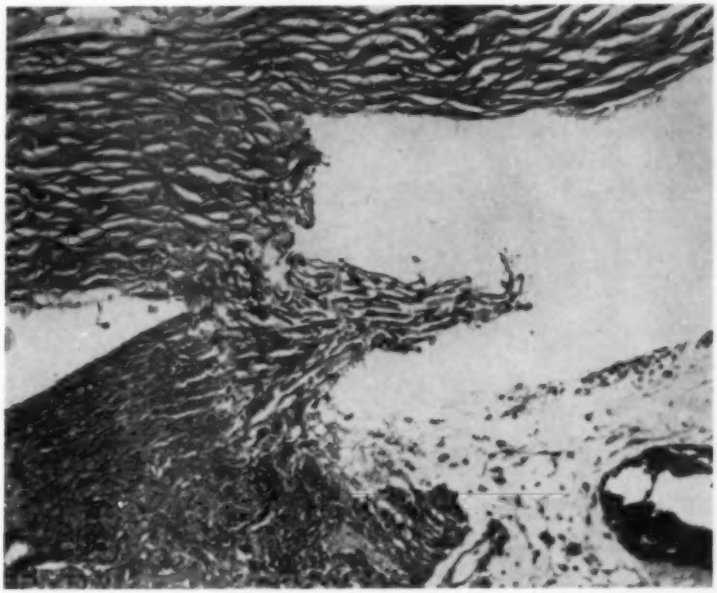


Fig. 2 (Dellaporta). Similar anatomic result of incomplete trabeculodialysis as in Figure 1.

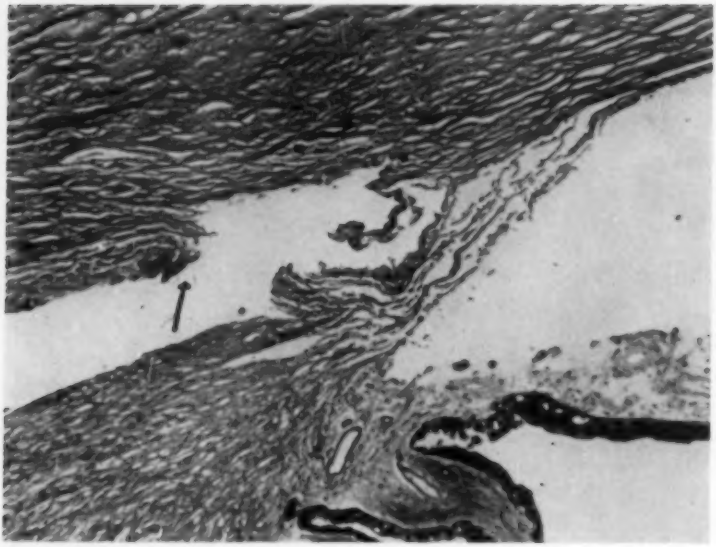


Fig. 3 (Dellaporta). Incomplete posterior trabeculodialysis where the corneoscleral trabeculum remained attached to its anterior attachment at Descemet's membrane while ciliary body and the base of the corneoscleral trabeculum had been separated. In this case the aqueous humor will not have free access to the outer wall of Schlemm's canal. Arrow indicates the posterior end of Schlemm's canal. Section derives from random segment of an eye quadrant treated by posterior trabeculodialysis.

it would be technically almost impossible to obtain consistently the desired anatomic effect.

An incomplete anatomic result due to a too short sweep of the blade into the anterior chamber is more unlikely to happen in posterior trabeculodialysis because in this method the exact position of the blade before withdrawing it is visualized through the cornea. However, an incomplete posterior trabeculodialysis was seen histologically in some slides of this series. The corneoscleral trabeculum remained adherent to its anterior attachment at Descemet's membrane even though ciliary body, scleral spur and the base of the corneoscleral trabeculum had been separated from the sclera (fig. 3). In such a case in spite of the anatomical formation of a subchoroidal cleft there would be no free communication between anterior chamber and Schlemm's canal or between anterior chamber and subchoroidal space. Thus, the aqueous humor would not reach the outer aspect of Schlemm's canal. These variations of incomplete anterior or posterior trabeculodialysis were observed histologically in this series only in sections which derived from random segments of some eye quandrants.

Comparison of anatomic results

Table 1 shows the anatomic results obtained with anterior or posterior trabeculodialysis using either the original angulated spatula or the thin angulated spatula mentioned previously.[1]

Table 1 indicates that the total percentage of successes, that is, anatomic opening of Schlemm's canal (figs. 4A and 4B) is almost identical in the two operations, being 95 percent in anterior trabeculodialysis and 94 percent in posterior trabeculodialysis.

The results obtained with the original angulated spatula in the operation of anterior trabeculodialysis were consistent enough to

TABLE 1

RESULTS WITH ANTERIOR OR POSTERIOR TRABECULODIALYSIS

Instrument Used	ATD		PTD	
	No. of Operations	No. of Successful Operations	No. of Operations	No. of Successful Operations
Original angulated spatula	52	49 (94%)	32	28 (87.5%)
Thin angulated spatula	6	6 (100%)	31	31 (100%)
Total	58	55 (95%)	63	59 (94%)

consider this instrument suitable for the successful performance of this procedure so that further investigations were considered superfluous.

Out of the 32 operations of posterior trabeculodialysis performed with the original angulated spatula, 28 were successful, representing a success percentage of 87.5 percent which, although high, was not as good as that obtained in anterior trabeculodialysis. The reason for this difference in results is probably the fact that in posterior trabeculodialysis the corneoscleral trabeculum is protected by the prominence of the scleral spur because the blade of the spatula approaches it from behind. This is especially true when this prominence is higher than average and the edge of the blade is thick enough to glide over the scleral spur. In anterior trabeculodialysis the scleral spur does not interfere with the actual detachment of the corneoscleral trabeculum since the blade approaches the latter from the anterior chamber. Having in mind that posterior trabeculodialysis performed with a spatula whose blade was a rod, was consistently unsuccessful in previous experiments,[1] it was theorized that a blade thinner than that of

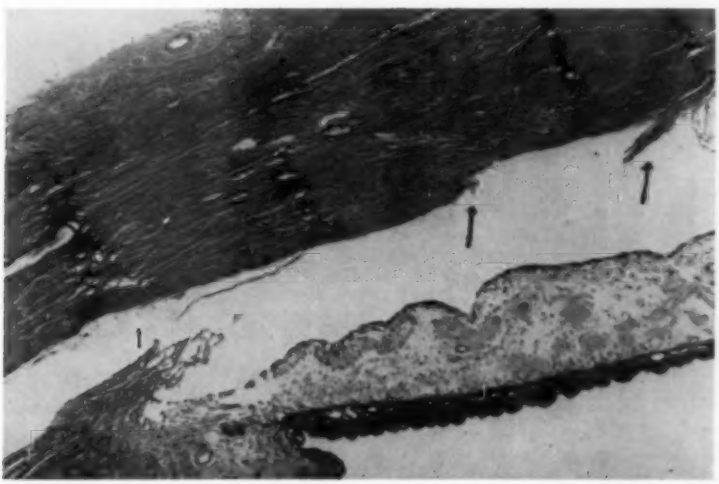


Fig. 4A (Dellaporta). Anatomic results of a typical trabeculodialysis. The corneoscleral trabeculum has completely detached and remains clinging to the anterior end of the ciliary body. Schlemm's canal (between arrows) has been converted into a shallow furrow.

Fig. 4B (Dellaporta). Higher magnification of Schlemm's canal from Figure 4A. Note intact endothelium lining the outer wall of Schlemm's canal.

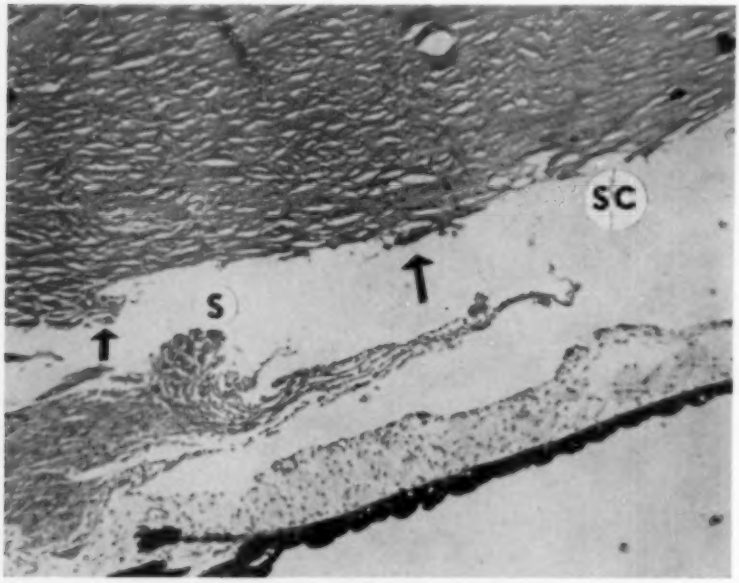


Fig. 5 (Dellaporta). Posterior trabeculodialysis performed with the thin angulated spatula. The scleral spur (s) has been separated together with the detached corneoscleral trabeculum. Schlemm's canal between arrows. Anterior to the latter the posterior corneal surface is denuded of Descemet's membrane and corneal endothelium and shows a rough surface (sc).

the original angulated spatula might be more successful in anatomically unfavorable cases. A new angulated spatula was constructed with a considerably thinner blade but not a sharp active edge. With it, 31 operations of posterior trabeculodialysis were performed and all proved to be anatomically successful (table 1). These improved results were, in my opinion, due to the fact that a prominent scleral spur will be detached together with the corneoscleral trabeculum by the thin edge of the new spatula (figs. 5 and 15) whereas the thick original angulated spatula would have glided over it. (With the thin angulated spatula six successful operations of anterior trabeculodialysis were performed and these cases were for statistical purposes added to the 52 anterior trabeculodialysis performed with the original angulated spatula since they showed almost identical anatomical results).

Histologic findings in the unsuccessful cases

The study of the histologic slides of these cases showed the following:

1. In two cases (both anterior trabeculodialysis) the cause of the failure was obviously poor technique. Some of the tissue of the separated ciliary body was found clinging to the scleral spur, indicating that owing to insufficient pull on the handle of the spatula or owing to improper position of the blade of the spatula during the sweeping movement, the active edge of the blade did not reach the corneoscleral trabeculum (figs. 6 and 7).

2. In one case (posterior trabeculodialysis) Schlemm's canal was deepset in the scleral tissue and could probably not be reached by the active edge of the blade.

3. In the remaining four cases (one anterior trabeculodialysis, three posterior trabeculodialysis) the cause of failure was histologically not obvious. It seems that anatomical variations, that is a relatively deepset Schlemm's canal in three cases and a relatively prominent scleral spur in the fourth case (fig. 8) played some role, but there were also signs of poor technique as described above under paragraph 1. It is my impression that with a thinner blade these

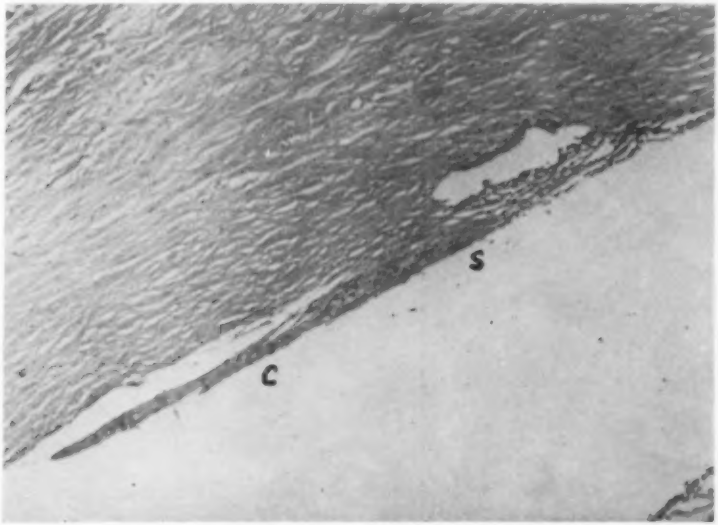


Fig. 6 (Dellaporta). Unsuccessful trabeculodialysis due to faulty operative technique. Some of the tissue of the ciliary body (c) remained attached to the scleral spur (s) indicating that the active edge of the blade did not reach the corneoscleral trabeculum.

Fig. 7 (Dellaporta). Unsuccessful trabeculodialysis as in Figure 6.

last four cases would have been successfully operated. This opinion is supported by the fact that many eyes with similar anatomic variations in the canal of Schlemm and the scleral spur, were successfully operated and by the excellent (100 percent) results obtained when using a thinner blade.

In summary, the cause of unsuccessful surgery might be listed in the following order of importance:

1. Inadequate instrument: (a) improper angulation of the blade of the spatula, (b) if the edge of the blade of the spatula is thick the corneoscleral trabeculum might not be detached.

2. Poor operative technique: (a) incor-

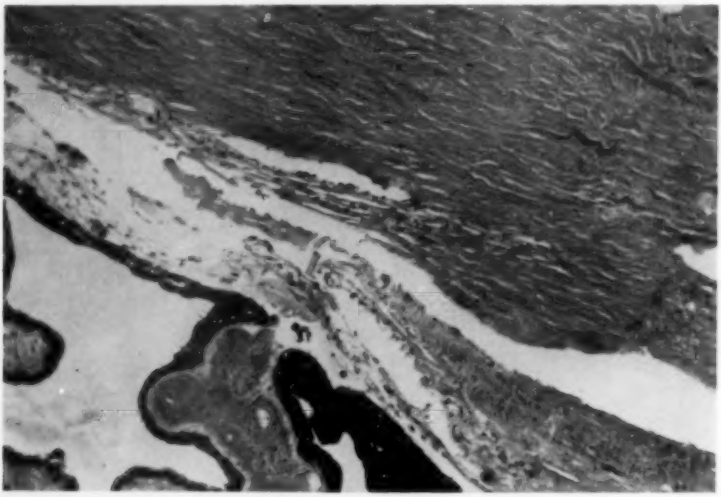


Fig. 8 (Dellaporta). Unsuccessful posterior trabeculodialysis probably due to the prominent scleral spur.

rect position of the blade of the spatula during sweeping movements; (b) insufficient pulling of the handle of the spatula during the sweeping movements.

3. Anatomic causes: (a) Prominent scleral spur; this would obviously interfere more in posterior trabeculodialysis than in anterior trabeculodialysis; (b) deepset Schlemm's canal in the scleral tissue. This would interfere equally in anterior trabeculodialysis or posterior trabeculodialysis.

My personal impression is that for a successful trabeculodialysis the most important single factor is the proper angulation of the blade of the spatula. Whatever the causes of failure were in these cases it seems that they do not occur often and specifically not twice in the same eye. All seven failures were observed in only one quadrant of any given eye, the other three quadrants showing a detached corneoscleral trabeculum. The 121 cases recorded here represent 121 quadrants belonging to 31 eyes (three quadrants could not be evaluated because of poor histologic technique). Therefore, in 23 out of a total of 30 eyes, the corneoscleral trabeculum was found detached in all four quadrants; in seven eyes the corneoscleral trabeculum had been separated in only three quadrants. This means that the second operation in any of these seven eyes was successful. Theoretically, one might then conclude that trabeculodialysis performed in one half of the circumference of the angle will open Schlemm's canal in any given eye.

Variants from the typical

In several specimens after anterior trabeculodialysis operation the detached corneoscleral trabeculum was found folded back into the subchoroidal cleft (fig. 9). This is easily explained by the sweeping of the blade of the spatula from the anterior chamber into the subchoroidal space. It is reasonable to assume that in a living eye the corneoscleral trabeculum owing to its normal elas-

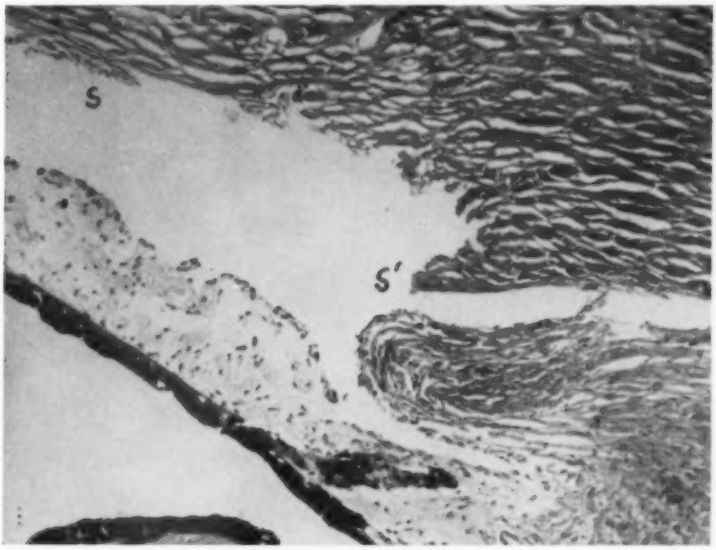


Fig. 9 (Dellaporta). Detached corneoscleral trabeculum folded back into the subchoroidal space caused by the sweep of the blade of the spatula after anterior trabeculodialysis. Outer aspect of Schlemm's canal (s-s') bare of endothelium.

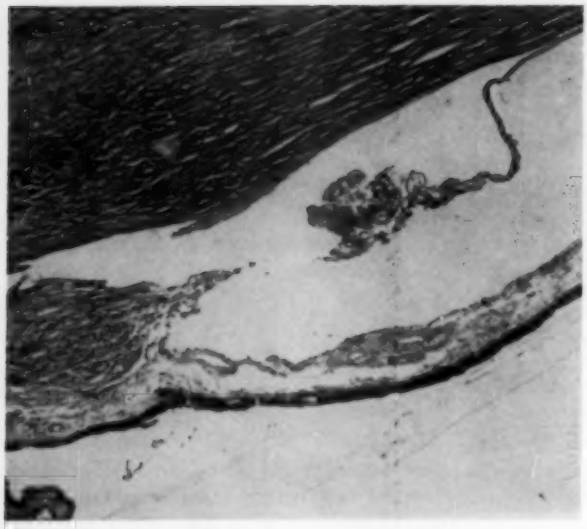


Fig. 10 (Dellaporta). Corneoscleral trabeculum detached from its bed and from the ciliary body but still clinging on to Descemet's membrane. The shallow Schlemm's canal is open, its endothelium intact. Condition after posterior trabeculodialysis.

ticity would return to its normal position. In occasional slides the corneoscleral trabeculum had detached from its base at the scleral spur, and was disconnected from the ciliary body but still clinging to its anterior attachment at the end of Descemet's membrane with Schlemm's canal open (fig. 10).

In contradistinction to the typical complete separation of the corneoscleral trabeculum found after trabeculodialysis some cases (anterior trabeculodialysis or posterior trabeculodialysis) showed the anatomic result of a trabeculectomy with the anterior and posterior ends of the trabeculae adherent to their original positions whereas the main part of the corneoscleral trabeculum was detached (fig. 11). In other cases the anatomic picture of trabeculotomy was seen with only

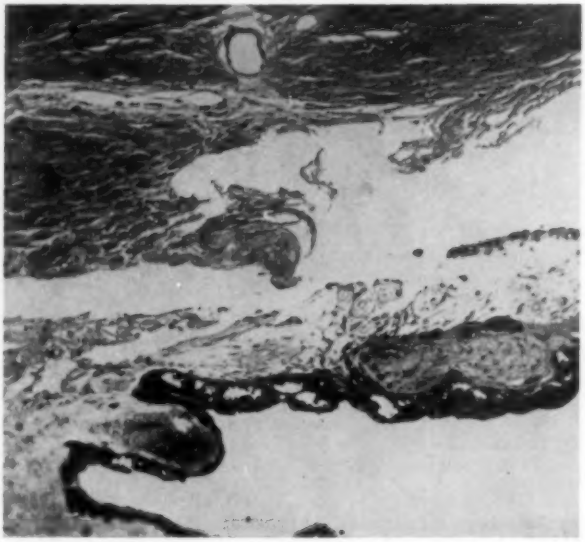


Fig. 11 (Dellaporta). Trabeculectomy. This condition is occasionally found after anterior or posterior trabeculodialysis. Since the cleft in the trabeculae is quite wide, it is unlikely to close and therefore the functional result of trabeculectomy would be identical to trabeculodialysis. In both cases the aqueous will have free access to the outer aspect of Schlemm's canal and the collector channels.

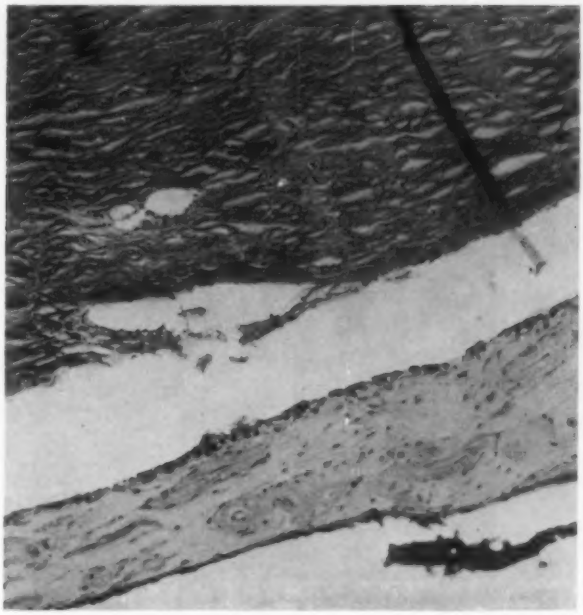
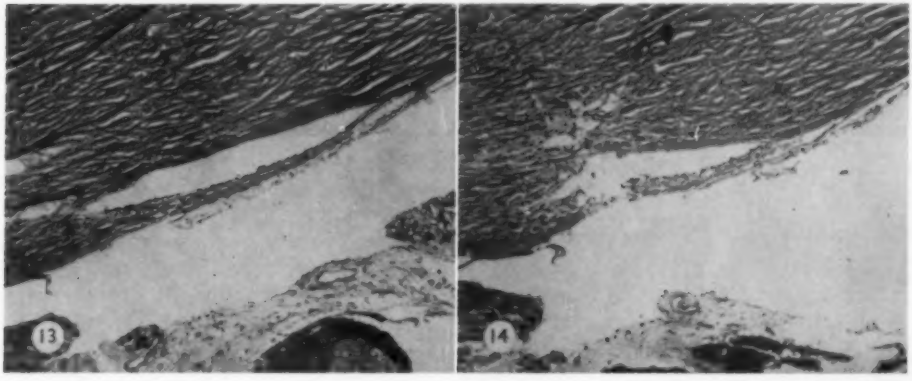


Fig. 12 (Dellaporta). Trabeculotomy occasionally found after posterior or anterior trabeculodialysis. It is probable that such a small slit of the trabeculae will close again by reapposition of the trabecular fibers and therefore the aqueous humor will not reach the outer aspect of Schlemm's canal.

a narrow slit along the trabeculae (figs. 12 and 14). These findings can be explained by anatomic variations of the form and position of Schlemm's canal and the covering trabeculae. This explanation is supported by the fact that in the serial sections of some specimens the transition of a still closed Schlemm's canal into a trabeculotomy and then into a trabeculodialysis could be followed histologically (figs. 13 and 14). It is obvious that if the trabecular slit is wide the functional result would be the same as in a typical trabeculodialysis whereas in case of the narrow slit Schlemm's canal will probably close again by reapposition of the trabeculae.



Figs. 13 and 14 (Dellaporta). These photographs show successive serial sections of the same specimen and illustrate the transition of a still closed Schlemm's canal (fig. 13) into a trabeculotomy (fig. 14). This indicates that occasionally, due to anatomical variations the corneoscleral trabeculum is not detached in the whole area of the operation.

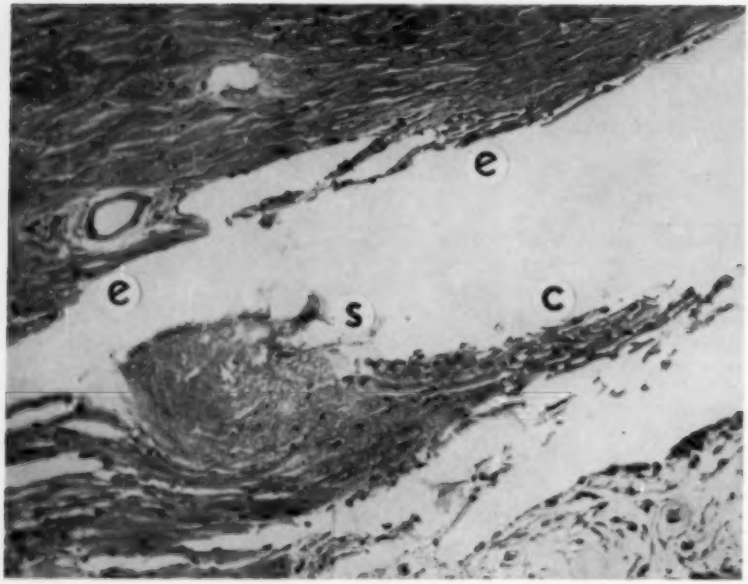


Fig. 15 (Dellaporta). Posterior trabeculodialysis with the thin angulated spatula. The scleral spur (s) has been detached together with the corneoscleral trabeculum (c). The inner lumen of Schlemm's canal is open, the outer lumen shows a trabeculotomy. Endothelium of Schlemm's canal is lining the inner wall of the outer lumen (e-e).

In some instances Schlemm's canal was found in the meridional sections to consist of two lumens the innermost of which was opened but the outermost still closed or very rarely showing a trabeculotomy. At first this gives the impression of an unsuccessful trabeculodialysis but the tracing of endothelium identical to that lining Schlemm's canal on the innermost wall (figs. 15, 16A, and 16B) of the second lumen proves the above mentioned anatomical result.

Anatomic side-effects of trabeculodialysis

Ciliary body

The very nature of trabeculodialysis makes it unavoidable that a limited cyclodialysis is produced at the operation. This cyclodialysis is for reasons already mentioned slightly wider in anterior trabeculodialysis than in posterior trabeculodialysis. It was not possible to evaluate anatomically the width of the cyclodialysis in many cases, the uvea having slipped from its postoperative position because the specimens were treated histologically after the fixed eye had been sectioned into four quadrants. It is my general impression however that after posterior trabeculodialysis the cyclodialysis was considerably less extensive than in anterior trabeculodialysis.

There is no reason to believe that this limited cyclodialysis will jeopardize in any way the anatomic or functional results of the trabeculodialysis. Theoretically this cyclodialysis might be of benefit because it will enable the relaxing ciliary muscle to pull the detached corneoscleral trabeculum posteriorly so that no reapposition of the latter over its natural bed would be possible and Schlemm's canal will remain open. Clinical experience indicates that the small subchoroidal cleft will close very soon postoperatively after it has served its purpose of preventing

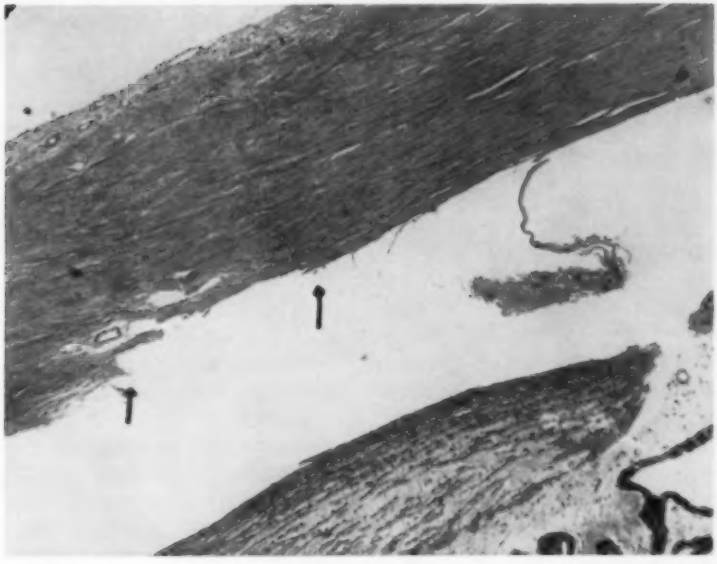


Fig. 16A (Dellaporta). Corneoscleral trabeculum detached from its bed and from the ciliary body and hanging on to Descemet's membrane. Schlemm's canal consists of three lumen, the innermost (between arrows) open, the middle showing a trabeculotomy and the outer being closed. See Figure 16B.

Fig. 16B (Dellaporta). Higher magnification showing the region of Schlemm's canal from Figure 16A. Note the endothelium lining the opened inner lumen.

reclosure of Schlemm's canal. From the point of view of the extent of the cyclodialysis produced in each operation posterior trabeculodialysis has to be given preference over anterior trabeculodialysis.

Descemet's membrane

Trabeculodialysis is bound to cause some damage to this membrane and the corresponding corneal endothelium. In order to obtain an objective picture of the extent of the damage done to Descemet's membrane by these two methods and by the different spatulas used the width of the detachment of Descemet's membrane of each serial section of each specimen was tabulated and the results computed.

The average width of the detachment of Descemet's membrane was: In 55 successful anterior trabeculodialysis operations, 1.2 mm.; in 59 successful posterior trabeculodialysis operations 1.9 mm. In 31 successful posterior trabeculodialysis operations with a thin angulated spatula, 1.8 mm. In the last 12 successful posterior trabeculodialysis operations with the original angulated spatula, 1.8 mm.; in the last 12 successful posterior trabeculodialysis operations with the thin angulated spatula, 1.2 mm.

This compilation indicates:

1. In anterior trabeculodialysis Descemet's membrane was slightly less damaged than in posterior trabeculodialysis.

2. The damage to Descemet's membrane in posterior trabeculodialysis was almost identical using the orginal angulated spatula or the thin angulated spatula.

3. The last 12 successive cases of posterior trabeculodialysis performed with the original angulated spatula and the last 12 successive cases performed with the thin angullated spatula show that the latter used with skill and caution causes less damage than the original angulated spatula and not more than that caused in anterior trabeculodialysis.

From clinical experience we know that operative detachment of Descemet's membrane of this or even higher degree which occurs occasionally in the various types of cyclodialysis do not cause permanent damage to the cornea.

Sclera

It seems that in anterior trabeculodialysis the active edge of the blade of the spatula first separates he corneoscleral trabeculum from its attachment to Descemet's membrane and then detaches it from the scleral spur. It is probable that the blade in many cases instead of gliding over the scleral spur after the detachment of the corneoscleral trabeculum engages in the small furrow formed by Schlemm's canal and the scleral spur, and splits the scleral fibers which terminate there from the main scleral body. This scleral split was found in 29 out of 55 operations of anterior trabeculodialysis and is usually a fraction of a millimeter long, (fig. 17) but in a few instances deriving mostly from the first operations one can see considerable scleral splitting as shown in Figure 18.

In the great majority of cases it is logical to assume that the short split will heal very soon without untoward consequences. It is evident that posterior trabeculodialysis by its very nature will not have a similar effect.

As far as this side-effect is concerned posterior trabeculodialysis is the obvious choice over anterior trabeculodialysis.

In eight cases out of a total of 31 posterior trabeculodialysis operations performed with the thin angulated spatula the inner surface of the cornea adjoining the anterior end of the corneoscleral trabeculum was not only denuded of Descemet's membrane and endothelium but also scratched as if treated with a knife (fig. 5). It is difficult to predict whether this limited injury will heal quickly without consequence or will cause a permanent or long-lasting edema of the corneal stroma with the known undesirable aftereffects. Since these changes were not found in anterior or in posterior trabeculodialysis operations performed with the original angulated spatula they have to be attributed to

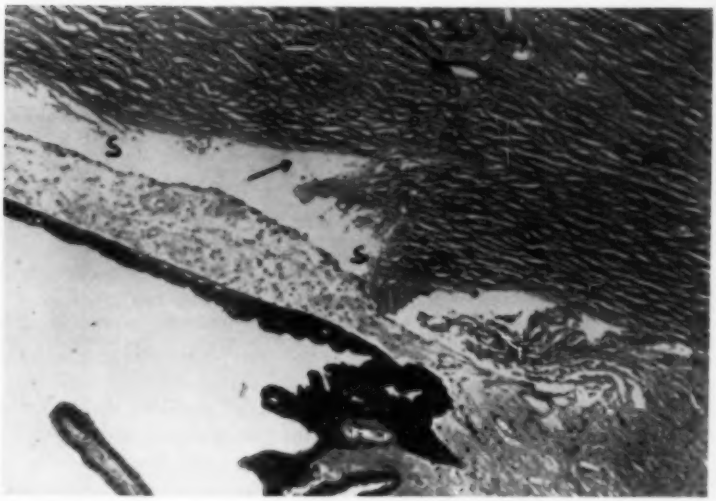


Fig. 17 (Dellaporta). Anterior trabeculodialysis. Open Schlemm's canal (ss). Posterior to it the displaced corneoscleral trabeculum. Small split (arrow) of the scleral fibers at the scleral spur. The outer aspect of Schlemm's canal is bare of endothelium.

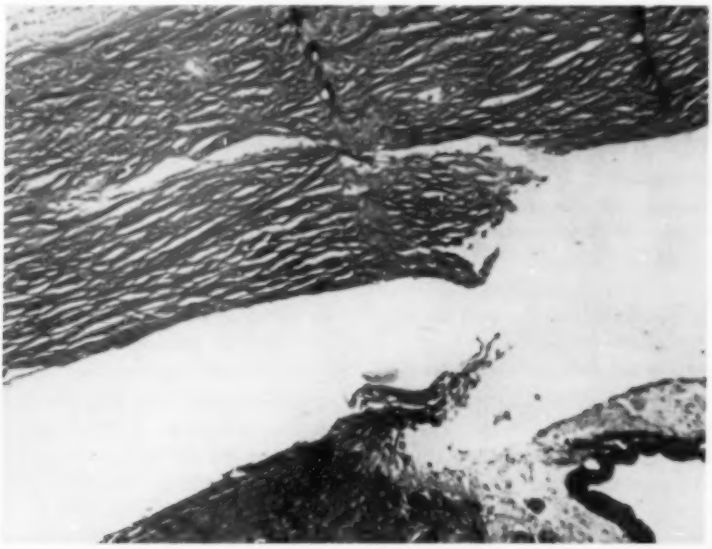


Fig. 18 (Dellaporta). Similar but longer split of scleral fibers at the scleral spur as in Figure 17 after anterior trabeculodialysis. Only the posterior half of Schlemm's canal is visible. Detached corneoscleral trabeculum hanging on the ciliary body.

TABLE 2

DAMAGE TO ENDOTHELIUM OF SCHLEMM'S CANAL

	−	±	+	‡
ATD (55 operations)	15	21	19	0
Total	36		19	
PTD (59 operations)	6	8	30	15
Total	14		45	

(−) means less than one half of the endothelium of Schlemn's canal intact.
(±) means one half of the endothelium intact.
(+) means more than one half of the endothelium intact.
(‡) means endothelium intact.

A similar comparative study between the operations of PTD performed with the original angulated spatula and those performed with the thin angulated spatula, showed no difference in the damage done to the endothelium of Schlemn's canal.

the thinner edge of the thin angulated spatula.

ENDOTHELIUM OF SCHLEMM'S CANAL

The changes occurring in the endothelium which lines Schlemm's canal and the collector channels are possibly of importance. Therefore an objective picture of the damage done to this tissue by the different types of trabeculodialysis operations using different spatulas was attempted. Each serial section of each specimen was tabulated and the computed results are shown in the following Table 2.

Table 2 shows:

1. Fifteen out of 55 operations of anterior trabeculodialysis showed considerable damage of the endothelium of Schlemm's canal whereas only six out of 59 operations of posterior trabeculodialysis showed an equal extent of damage.

2. Of 55 anterior trabeculodialysis operations 19 showed the endothelium in good condition, whereas out of 59 posterior trabeculodialysis operations 30 showed the endothelium in good condition and 15 in excellent condition.

The conclusion is therefore that damage to the endothelium of Schlemm's canal occurs more often and is more extensive in anterior trabeculodialysis than in posterior trabeculodialysis.

The importance of the endothelium is stressed by the fact that after detachment of the corneoscleral trabeculum from its bed, Schlemm's canal opens and the aqueous humor is in continuous contact with the endothelium which lines the outer aspect of Schlemm's canal and the collector channels. The clinical value of trabeculodialysis in increasing the facility of outflow will be determined finally by the behavior of this structure under the continuous influence of the aqueous humor. The aqueous humor is the normal medium of the trabeculae and of Schlemm's canal so that theoretically one would not expect reactive changes in the intact endothelium by which the collector channels might close. On the other hand, if the endothelium is stripped away through the operation, reactive changes from the denuded scleral tissue or damaged endothelium are possible and might close the openings of the collector channels. It is quite possible however, that this endothelium might posses similar regenerative capabilities as the corneal endothelium, in which case it will soon heal over the abraded areas.

As far as the operative damage to the endothelium of Schlemm's canal is concerned posterior trabeculodialysis is to be preferred over anterior trabeculodialysis.

CONCLUSIONS

From this comparative study the following may be concluded:

1. Anterior trabeculodialysis was successful in this series in 95 percent of the cases out of a total of 58 operations. The advantages of this method are the high rate of successes and the minor detachment of Descemet's membrane. A disadvantage is the coincidental damage done to the endothelium of Schlemm's canal found in many cases.

2. Posterior trabeculodialysis performed with the thin angulated spatula was in this series successful in 100 percent of the cases out of a total of 31 operations. Advantages of this procedure are the excellent rate of successes, the minor and infrequent damage to the endothelium of Schlemm's canal, and the minor coincidental cyclodialysis. A disadvantage is the occasional damage to the inner surface of the cornea which might cause corneal edema.

3. Posterior trabeculodialysis performed with the original angulated spatula* in 32 cases was successful in 87.5 percent of the operations. In spite of the slightly lower rate of successes this methods is, in my opinion, the most satisfactory. The advantages are the minor and infrequent damage to the endothelium of Schlemm's canal, the absence of damage to the inner surface of the cornea and the narrow coincidental cyclodialysis.

490 Post Street (2)

* An improved experimental model of the trabeculodialysis spatula can be obtained from Parsons Optical Company, 518 Powell Street, San Francisco 2.

Reference

1. Dellaporta, A.: The surgical separation of the corneoscleral trabeculum from its bed: I. Anterior trabeculodialysis, Am. J. Ophth., **47**:783, 1959; II. Posterior trabeculodialysis. Am. J. Ophth., **48**:15, 1959.

Discussion

Dr. Karl W. Ascher (Cincinnati): I want to congratulate the speaker for his excellent work. Performed on the living eyes his operation will combine the effect of a supraciliary cleft with that of opening the canal of Schlemm, giving access to its outlets. These should not be called collectors since they do not collect but distribute the intraocular fluid. There is still an open question, namely whether the resistance to this fluid elimination resides in the trabeculum or in the area beyond the canal. Perkins showed that the pressure drop between anterior chamber and the canal amounts to only 10 percent of the whole difference between intraocular pressure and the episcleral veins, that means 90 percent of the resistance is located beyond the outer wall of Schlemm's canal. In glaucomatous eyes, the distribution of resistance may be quite different. This remark is not meant to minimize the value of the presentation which is very stimulating indeed.

OBSERVATIONS ON THE LENS PROTEINS ALPHA AND BETA CRYSTALLIN*

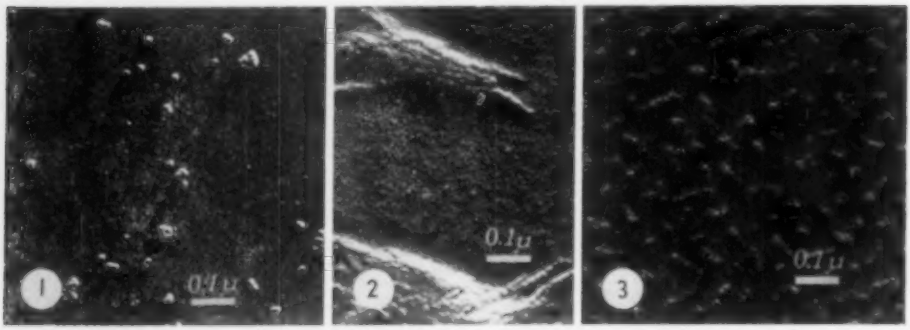
Robert A. Resnik, Ph.D., Theodor Wanko, M.D., and Mary Ann Gavin, M.S.
Bethesda, Maryland

Introduction

The soluble lens proteins have been investigated by means of various chemical techniques. [1,2] In electron micrographs of lens fibers, they have been correlated with discrete structures seen in the cytoplasm.[3,4]

The purpose of the present study was the electron microscope observation of isolated alpha and beta crystallins. The information

* From the Ophthalmology Branch, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare.



Figs. 1, 2, and 3 (Resnik, Wanko and Gavin). *Figure 1:* 0.1 percent alpha crystallin in water after lyophilization. Spherical particles, isolated and in smal aggregates, are seen. (Preshadowed replica, approximately ×70,000.) *Fig. 2:* 0.002-percent alpha crystallin in Na veronal and NaCl. Two groups with elongated structures in a side by side alignment are depicted in this field. (Spray on grid, approximately ×70,000.) *Fig. 3:* 0.01-percent beta crystallin in water. Spherical units are associated in small clumps and short chains. (Preshadowed replica, approximately ×70,000.)

obtained with this method about these two groups of proteins is discussed with reference to their known characteristics.

Materials and Methods

The soluble lens proteins were obtained, free of cellular elements, by centrifuging calf lens homogenates in 0.15 *M* sucrose for 10 minutes at 20,000 × g in a Servall centrifuge and then for two hours at 105,000 × g in a Model L Spinco preparative ultracentrifuge. Samples of alpha and beta crystallin were isolated from this solution after they had been resolved by moving boundary electrophoresis. This was carried out at 0.9°C. in a pH 8.3 veronal buffer with an ionic strength of 0.1. Alpha crystallin was also obtained by precipitation at pH 5.0 by adding alcohol to a concentration of 12 percent. From the resulting supernatant, the beta crystallins were isolated by the method of François.[2] The entire beta crystallin fraction, as well as the fastest and slowest migrating components obtained from electrophoresis, were used. Protein concentrations were estimated from the optical density of solutions at 280 and 260 mμ.[5] Ultracentrifuge experiments were performed in a Spinco Model E analytical ultracentrifuge. Electrophoresis was done in a Model H Spinco electrophoresis apparatus.

For electron microscopy solutions of alpha and beta crystallins were prepared in serial dilutions from 0.1 to 0.0001 percent, as single drops on grids, sprays on grids, and as pre-shadowed replicas from freshly cleaved mica. The following solvents were used: 0.15 *M* ammonium formate, 0.075 *M* sodium veronal and 0.025 *M* sodium chloride at pH 8.3 and distilled water. In addition to alpha crystallin isolated by electrophoresis, an aqueous solution of a lyphilized preparation was also investigated. All specimens were washed with distilled water except where ammonium formate or water was the solvent. Controls were prapared by processing the solvents alone. Specimens were shadowed with platinum at an angle of tangent 0.1250 and then coated with a thin carbon film. Electron micrographs were recorded at a magnification of 15,500 × and photographically enlarged ×4.5.

Results

Alpha crystallin

Preparations containing this protein exhibit a considerable variation in appearance, depending upon the solvent. Alpha crystallin in water, after lyophilization (fig. 1), and in ammonium formate always shows particles of an approximately spherical shape and different diameters. Aggregates of a num-

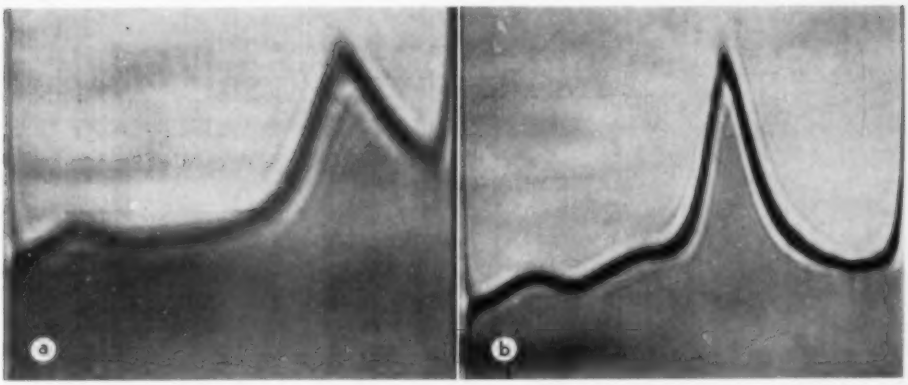


Fig. 4 (Resnik, Wanko and Gavin). Sedimentation patterns of alpha crystallin at pH 7.3 after 32 minutes at 59,780 rpm. (a) In 0.1 *M* NaCl. (b) In water.

ber of the individual units are seen frequently, even at the lowest concentrations. Elongated particles with an indication of a substructure can be demonstrated in specimens prepared in sodium veronal-sodium chloride only. They appear predominantly as filaments in a side-by-side arrangement (fig. 2). Isolated filaments with an average diameter of 32 Å and of variable length are found occasionally.

Beta crystallin

Each preparation of this fraction contains spherical particles which frequently are grouped in monolayered sheets. In some instances, as in Figure 3, where the material is adequately dispersed, individual, small spheres with an average diameter of 22 Å are arranged in small clumps and short chains.

Discussion

Physical-chemical data indicate that alpha crystallin is an elongated molecule of 30-70 Å in diameter.[6] It was evident from the foregoing observations that alpha crystallin retains a structure comparable to these calculated values only under certain conditions.

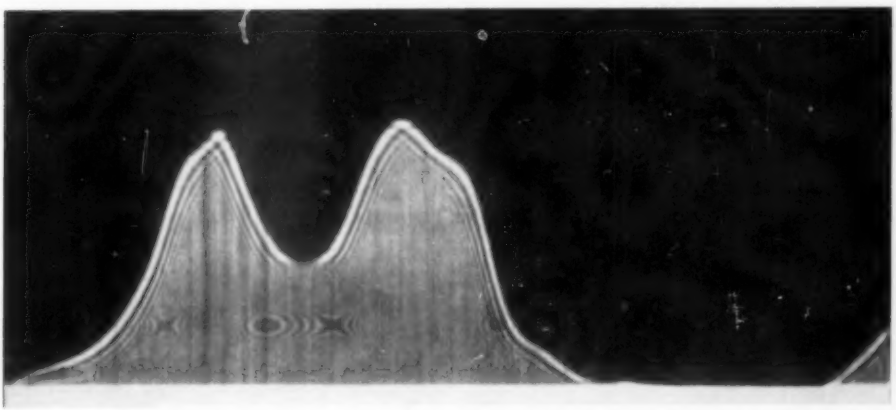


Fig. 5 (Resnik, Wanko and Gavin). Moving boundary electrophoresis pattern of the beta crystallin fraction of calf lens. Ascending limb after 9.5 hours.

The apparent variations of this protein in the different solvents employed can be explained by its alteration at low ionic strength.[6] It has been established that changes occur in alpha crystallin at low ionic strength over a wide pH range. Such a change is depicted in Figure 4 were the ultracentrifuge pattern in 0.1 *M* sodium chloride, pH 7.3 and that in water at pH 7.3 are compared. Since these alterations may be reversed by increasing the salt concentration, trace amounts of salts might conceivably influence the appearance of this molecule.

A group of about six proteins constitutes the beta crystallin fraction (fig. 5). The physical-chemical data presently available are insufficient for a valid correlation with the electron microscopic results. Therefore, the only conclusion possible is, first, that beta crystallin appears spherical with a diameter 10 Å less than alpha crystallin. Secondly, this configuration persists regardless of the solvent. It occurs whether the fastest or the slowest moving components alone are used or whether the entire fraction is employed.

Ophthalmology Branch.

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PSYCHOPATHOLOGY IN ADULTS WITH UVEITIS*

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This study was designed to determine whether psychopathology is associated with uveitis in adult patients. Such an association has already been demonstrated in glaucoma,[1] and has been suspected in uveitis.[2]

Procedure

This study began with 59 consecutive adult patients with uveitis of unselected types. Each patient was interviewed by a medical caseworker of the Department of Ophthalmology. (The results of these interviews will form the basis of a separate report.) At a subsequent visit a booklet form of the Minnesota Multiphasic Personality Inventory was administered to each patient by a social worker working under the supervision of a staff psychologist.

The Minnesota Multiphasic Personality Inventory is a well standardized psychologic test consisting of 550 statements concerning habits, attitudes, emotions, thoughts, fears and general health, to which the patient responds by indicating whether each statement for him is true or false. Responses

* From the Departments of Ophthalmology and Psychiatry, Indiana University School of Medicine. This study was supported in part by a grant from the Knights Templar Eye Foundation and by Grant B 1231 C from the National Institutes of Neurological Diseases and Blindness.

indicative of psychopathology are grouped into eight scales, each representing symptoms commonly found in persons with certain basic psychiatric illness. These illnesses are depression, hypochondriasis, and hysteria (the neurotic triad); psychasthenia, schizophrenia, and paranoia (the psychotic triad); and hypomania and psychopathic personality. Norms for persons without emotional illness are well established,[3,4] and the diagnosis of psychopathology from the scales is based on these norms.

Eleven of the 59 patients were not used for the following reasons: five had vision too poor to co-operate, five failed to co-operate adequately, and one was not studied because of an administrative error. This provided 48 patients for this report.

Results

In Table 1 are descriptive data from the uveitis survey of the 48 adult uveitis patients and in Table 2 the criteria used in the grading of the severity of involvement.

An abnormal score on any scale of the Minnesota Multiphasic Personality Inventory is one which is two standard deviations or more above the average attained by normal individuals.[4] Such an abnormal score will occur in 2.5 percent of a random unselected sample. Therefore in our sample of 48, we would expect 1.2 individuals to score pathologically high if tested on only one scale, and 1.2 times eight or 9.6 individuals to score high on any one of the eight scales. The estimate of 9.6 assumes that the scales are not correlated which is not true, so 9.6 must be considered a maximum figure.

On a basis of these calculations, our results deviate significantly from the chance expectation. Twenty-one of the 48 individuals obtained a pathologic score on one or more of the scales (corrected chi-square of 15.47; $p = <0.0001$).

The Minnesota Multiphasic Personality Inventory also contains two scales (K and L) the function of which is to single out individuals who are being evasive or defensive in responding to the test. It is of interest that of the 27 patients who did not show any pathology on the Minnesota Multiphasic Personality Inventory 20 had elevated K or L scales, or both. Only four of the 21 patients with pathologic Minnesota Multiphasic Personality Inventory scales also showed these evidences of evasiveness. A total of 15 patients without psychopathologic Minnesota Multiphasic Personality Inventory scales had K scale scores of 1.0 SD or more above the normative mean for this scale, as compared with an expected frequency of 7.68 (16 percent) for the entire sample of 48. A corrected chi-square is 11.72 ($p < 0.001$).

There thus seems little doubt that most of the 27 patients in our sample avoided obtaining pathologic Minnesota Multiphasic Personality Inventory scale scores by defensiveness or evasiveness. In addition, we should mention the five patients who failed to complete the tests, since failure to co-operate is often another means of evasion. These findings suggest that the degree of psychopathology in our uveitis cases is probably greater than we have measured it and our p value was <0.0001.

Nine patients obtained a pathologic score on the Hysteria and 11 on the Depression Scales, while six had pathologic scores on the Hypochondriasis Scale. When compared to the expected frequency, the smallest of these three (Hypochondriasis) gave a chi-square of 15.85 ($p < 0.0001$). In regard to each of the other scales we found no more than two patients with pathologic scores, a frequency which could have been due to chance ($p > 0.05$).

Thirteen of the 26 females (50%) in the study obtained at least one pathologic MMPI scale as compared to eight of the 22 males (36 percent). The difference between the frequencies of pathology for the sexes yields a chi-square of 0.44; ($p = 0.50$) which is not significant and indicates that *sex* is not correlated with psychopathology in uveitis patients.

An analysis was made of the relationship

TABLE 1

DESCRIPTION OF THE SAMPLE OF 48 ADULT UVEITIS PATIENTS USED IN THIS STUDY

Patient Number	Race & Sex	Site	Type	Grade of Severity†	Possible Etiology
1	WM	Both	Gran.	3	Toxoplasmosis
2	WM	Both	Gran.	4	Toxoplasmosis
3*	CF	Both	Gran.	4	—
4	WF	Ant.	Nongran.	3	Rheu. Arthritis
5	WM	Both	Gran.	1	—
7	WM	Both	Gran.	3	Toxoplasmosis
8*	WM	Post.	Gran.	3	Toxoplasmosis
10*	WF	Post.	Gran.	2	—
11*	WM	Ant.	Gran.	2	—
12*	WF	Post.	Gran.	2	—
13	WF	Both	Gran.	3	—
14	WF	Ant.	Gran.	1	—
15*	WF	Both	Gran.	3	Toxoplasmosis
16	CM	Ant.	Gran.	1	Tuberculosis
17	WM	Ant.	Gran.	1	—
18	WF	Post.	Gran.	1	Histoplasmosis
20	WF	Post.	Gran.	2	Tuberculosis
21*	WF	Ant.	Nongran.	1	—
23	WM	Post.	Nongran.	3	—
25*	CF	Post.	Gran.	2	—
26*	WM	Ant.	Nongran.	3	Tuberculosis
28	WM	Post.	Gran.	3	—
29	WM	Both	Gran.	1	—
30*	WM	Post.	Gran.	2	Tuberculosis
31*	WM	Ant.	Nongran.	3	—
32*	WF	Ant.	Nongran.	3	—
33	WF	Ant.	Gran.	2	Tuberculosis
34	WF	Both	Gran.	3	—
35*	CF	Ant.	Nongran.	2	—
36*	WF	Ant.	Gran.	1	Heterochromic cylitis
37*	WF	Post.	Gran.	3	—
38	WF	Post.	Nongran.	1	Histoplasmosis
40	WM	Post.	Gran.	2	Tuberculosis
41*	WF	Ant.	Gran.	2	—
42*	WF	Both	Gran.	4	Toxoplasmosis
45	WF	Both	Nongran.	2	—
47	WM	Ant.	Gran.	2	Heterochromic cylitis
48	WF	Post.	Gran.	0	—
49	WM	Both	Gran.	2	Toxoplasmosis
50*	WM	Post.	Gran.	3	—
51	WF	Both	Gran.	3	—
52*	WM	Post.	Gran.	2	—
53	WF	Ant.	Nongran.	3	—
54	WM	Ant.	Nongran.	1	—
55*	WF	Both.	Gran.	3	Toxoplasmosis
57	WM	Both.	Gran.	4	—
60	WF	Both	Gran.	3	—
61*	WF	Both.	Gran.	2	—

* Patient had at least one psychopathologic MMPI scale.
† 4 is most severe.
There were originally 59 uveitis patients+2 controls=61.

between *site of the involvement* (anterior, posterior, both) and psychopathology scores. Trichotomous analysis gave a chi-square of 2.24; ($p < 0.30$). Since involvement of the anterior segment in chorioretinitis is usually due to a spill-over from[5] or a reaction to[6] the posterior inflammation, we then grouped patients with involvement of both segments with those with involvement of the posterior segment alone and constrasted them with the patients with anterior involvement alone. This dichotomous analysis gave a chi-square of 0.10 or a p of 0.75. We may thus conclude that site of involvement is not correlated with psychopathology in uveitis patients.

The relationship between the *type of in-*

TABLE 2

CRITERIA FOR GRADING OF SEVERITY OF UVEITIS

	Grade
Anterior Uveitis	
Flare, cells, and keratic precipitates of mild degree	1
Flare, cells, and keratic precipitates of moderate degree	2
Vision 20/200 or worse and/or flare, cells and keratic precipitates or moderately severe degree	3
Vision worse than 20/200 and flare, cells, and keratic precipitates of severe degree	4
Posterior Uveitis	
Small chorioretinitis with little or no vitreous haze and little or no reduction in visual acuity	1
Moderate-sized chorioretinitis with some vitreous haze and some reduction in visual acuity	2
Vision of 20/200 or worse with moderate-sized chorioretinitis and moderately severe vitreous clouding. Usually unable to see the fundus	3
Vision worse than 20/200 to no light perception. Severely cloudy media. Includes cases with secondary cataract and phthisis bulbi	4

flammation (granulomatous or nongranulomatous) and psychopathology revealed a chi-square value of 0.00, giving a p of 0.99 which indicates that the type of inflammation is not correlated with psychopathology.

The correlation between *severity of eye involvement* and of psychopathology was determined by dividing the patients into those cases with at least one pathologically high MMPI scale and those cases with normal records, and computing a biserial correlation coefficient with the ratings of severity of eye involvement ranging from 0 to 4. The mean severity rating for the normal group is 2.15 while that for the patients with psychopathology is 2.48. This biserial coefficient was 0.21 which does not differ significantly from 0 and thus the severity of eye involvement is not correlated with psychopathology.

Seven (37 percent) of the 19 cases in which the *etiology of the uveitis* was diagnosed and 14 (48 percent) of the 29 cases in which the etiology was undiagnosed obtained pathologic Minnesota Multiphasic Personality Inventory scales. The difference is suggestive, but not significant (corrected chi-square $= 0.23$, $p = .62$).

COMMENT

The data clearly show a significant incidence of psychopathology in our adult patients with uveitis.

While a correlation does not indicate causation, the data suggest that psychopathology could be a factor underlying the development of uveitis.

The absence of relationship between psychopathology and severity of eye involvement is suggestive of the role of psychogenic factors in the pathogenesis of uveitis. If uveitis caused the psychopathology we would expect psychopathology to occur more frequently in those patients with the severest uveitis but this was not true.

The lack of correlation of the severity of psychopathology with sex, type of inflammation (granulomatous or nongranulomatous), site of involvement (anterior, posterior or both), severity of involvement, or etiologic diagnosis would indicate that psychic factors may be involved in any case of uveitis and not just in certain types.

It is our tentative hypothesis that two factors operate concurrently to result in uveitis: (1) and agent (such as a bacterium, virus, or allergen) and (2) some type of external stress or internal psychopathology. It is to be emphasized, however, that this is a tentative hypothesis which must be tested by further study.

Another possible explanation of the data is that both psychopathology and uveitis may have a common organic origin, and this possibility is supported by the recent experimental studies on the simultaneous production of encephalomyelitis and uveitis by the same allergen.[7,8]

CONCLUSION

In a study of 48 adults, uveitis and psychopathology were found to be related. The significance of this relationship is not yet

understood, and, therefore, it should not be interpreted necessarily that psychopathology is involved in the pathogenesis of uveitis, since it is not known which is cause and which is effect or whether the relationship is due to some common cause. These results do suggest that further study of the relationship between uveitis and psychologic factors may prove fruitful.

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We wish to thank Mrs. Evelyn Dunbar, M.A., medical caseworker of the Department of Ophthalmology, for the administration of the Minnesota Multiphasic Personality Inventory test in this study.

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DISTRIBUTION OF RADIAL CONTRACTILE FORCES IN THE IRIS OF CATS*

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Chicago, Illinois

Among the contractile forces in the iris of mammals there is one capable of narrowing the distance between the pupil margin and the attachment of the root of the iris to the sclera. This force has been repeatedly demonstrated by stimulation of isolated iris sectors both in situ[2, 8, 11, 12, 22, 23, 25, 27, 32, 37, 42] and in vitro.[1, 3, 14, 15, 16, 17, 18, 19, 20, 21, 24, 26, 30, 32, 33, 34, 36, 41] However, the precise location in the iris of the structure giving rise to this force has never been established. It has simply been assumed that the force arose in the so-called "dilator pupillae," although this structure is not typically muscle in appearance.[9, 12, 13, 23, 25, 28, 29, 31, 35, 37, 38, 40, 42, 46] On the other hand typical muscular fibers extending in the radial direction have been described in both the sphincter pupillae and the ciliary muscles connecting the iris to the sclera.[28, 35] In view of the ambiguous histologic evidence, the crucial test for locating radial muscles in the iris is the physiologic one—the development of active tension in response to appropriate stimulation. It is the purpose of the present study to explore the distribution of radially-directed contractile forces in the iris of cats, using this physiologic test.

The eyes of cats were removed under nembutal anesthesia and the cornea was immediately cut away one and one-half millimeters behind the limbus (fig. 1). Following this procedure, the pupil assumes the shape of a 1.0 by 5.0-mm. oval. Slits one to 1.5-mm. long were cut through the thickness of the iris in the radial direction in three different areas: the sphincter area, root area and intermediate between them. The iris segment ex-

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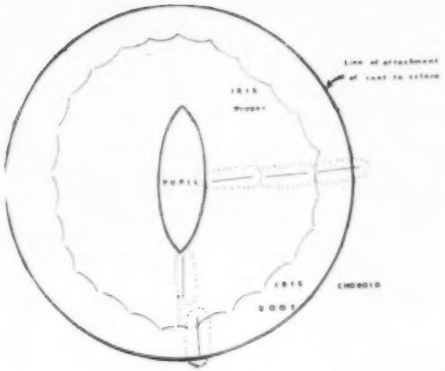


Fig. 1 (Apter). Cat iris with cornea and corneoscleral junction removed. Whole iris and its root, overlying the ciliary body, is exposed. Slits (solid radial lines) were cut through the iris in three locations in the lateral iris and in two locations at the apex of the pupil. The iris segment surrounding the slits was removed by cutting with a de Wecker scissors on the dotted line. Segments from the sphincter, middle and root portions of the iris are thus isolated.

tending 2.0 mm. around the slit was excised (fig. 1). The solid line is the slit and the dotted line is the outline of the excised segment. The excised segments were suspended horizontally between two hooks in a bath containing an artificial medium (fig. 2). A tris (hydroxymethyl) amino-methane buffered aerated mammalian Ringer's solution containing 200 mg. percent of glucose was used, commonly at about 15°C., where the responses of known muscle specimens were most active.[39] One hook could be manipulated laterally to induce stretch. The other hook was attached to the lower free end of a straight ribbon of spring steel, the upper end of which was firmly fixed. Displacement of the free end of the spring displaced a beam of light reflected from a mirror attached to the spring. This system was calibrated with weights over the range of 10 to 100 mg., the range appropriate to active forces developed by iris segments.

After the segment was placed over the hooks, it was stretched carefully by means of the adjustable support until a small resting force of 20 to 25 mg. was registered by the strain gauge. Stimulation consisted in adding mecholyl or adrenalin to the surrounding medium or in applying electrical stimulation via the two supporting hooks connected to a Grass stimulator. Each drug was applied in graded doses and the steady state tension induced by each dose was recorded to obtain a dose-response curve. For electrical stimulation the Grass stimulator supplied a 60-cycle alternating current of graded voltage.[5, 6, 7, 45] Here too, the steady state contractile force was measured and plotted against the stimulating voltage. The most important item in this apparatus is the segment of iris tissue. Its components must be known.

The anatomic constituents of each segment were regulated by careful selection of the areas for placing slits and by subsequent microscope examination of the excised segments. A diagrammatic cross-section of the iris (fig. 3) shows that the sphincter pupillae was present in the specimens taken from the portion of the iris nearest to the pupil. There are numerous circumferential fibers in this muscle and also some radial fibers in its spoke bundles. In the middle region there is only the dilator pupillae with alleged muscle fibers running—radially. His-

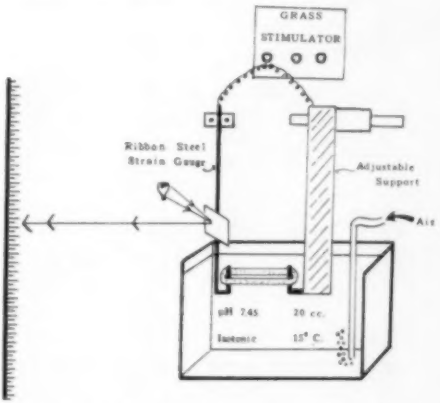


Fig. 2 (Apter). Iris segment is suspended on two platinum hooks placed through the slit. See text.

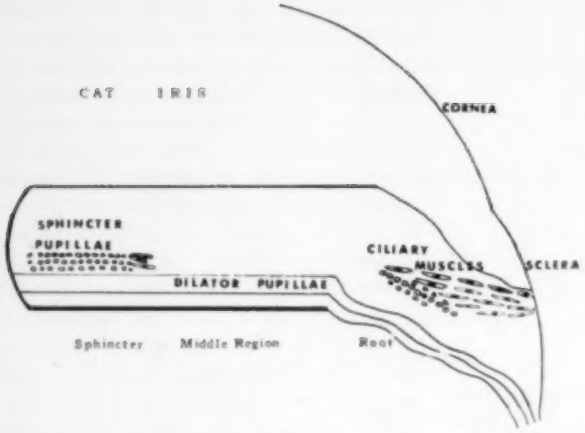
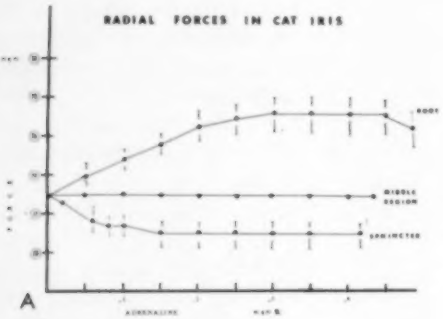
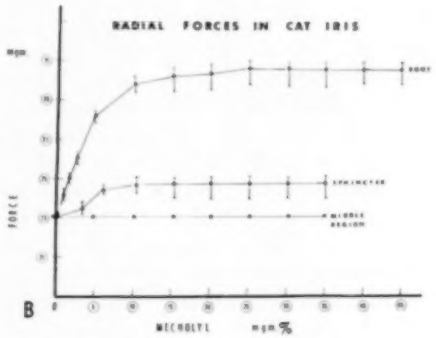
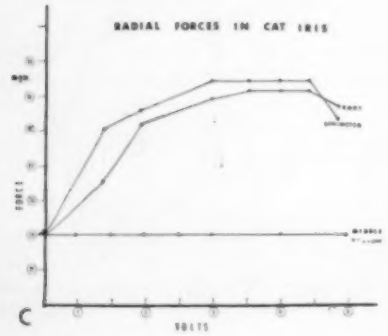


Fig. 3 (Apter). Schematic diagram of a cross-section of the lateral cat iris showing the arrangement of muscle fibers in the sphincter pupillae and ciliary muscles and the location of the so-called dilator pupillae.

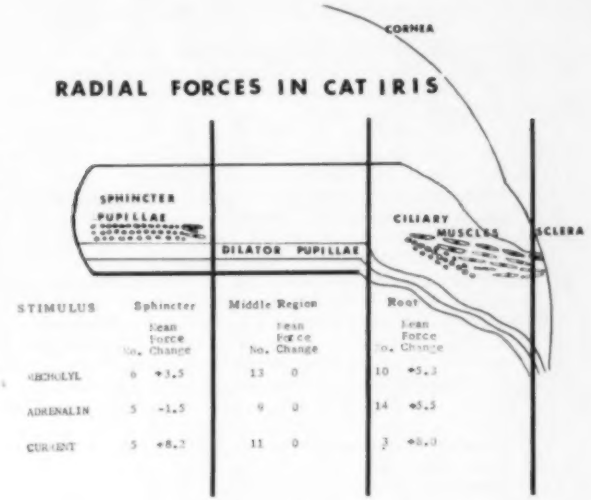
tologists maintain that the "dilator pupillae" has muscular characteristics only in this intermediate region of the iris although its two layers appear to be continuous with similar layers under the sphincter and in the ciliary body. In the root region the fiber bundles of the ciliary muscle are arranged circumferentially as well as radially. By placing radial slits in pupillary, root and intermediate portions of the iris, the contraction of radial fibers of sphincter, ciliary and dilator muscles were tested separately. What is more, the contractile power of the several muscles could be quantitatively compared since the







* Figs. 4A, 4B, and 4C (Apter). Relation between the strength of stimulus and the amplitude of active force developed by segments of iris. Each plot is a mean value. (See Figure 5 for number of specimens tested in each region.) Range of responses is shown except in the electrical stimulation curves in Figure 4C. In this instance the range of root responses overlaps the range of sphincter responses. The range for these two areas is of the same order of magnitude ($\pm$0.8 mg.) for electrical stimulation as for drug stimulation. In the middle region no responses to stimulation were measured in any of 33 specimens.



STIMULUS	Sphincter No.	Sphincter Mean Force Change	Middle Region No.	Middle Region Mean Force Change	Root No.	Root Mean Force Change
MECHOLYL	6	+3.5	13	0	10	+5.3
ADRENALIN	5	-1.5	9	0	14	+5.5
CURRENT	5	+8.2	11	0	3	+8.0

Fig. 5 (Apter). Summary of forces (in mg.) developed in segments of iris tissue to three kinds of stimulation. Active tension was registered only in areas that contain radial muscle fibers of the ordinary smooth muscle type. No tension was developed in segments containing the so-called dilator pupillae.

specimens were of similar size and all were tested at the same resting tension.[4, 6, 7, 10, 43, 44, 45]

Figure 4A shows the set of curves illustrating the dose-response relationship for changes in radial tension induced in each area by adrenalin. All specimens were set at an initial resting tension of 23 mg. In the sphincter region the tension dropped; in the root region the tension rose; and the middle area did not respond at all. Mecholyl (fig. 4B) and electrical stimulation (fig. 4C) gave similar dose-response curves with the plateau at maximal response.

The table in Figure 5 lists the maximal changes in radial tension in mg. induced by each drug and by electrical stimulation for each area of the iris. Please keep in mind that these are in the radial direction alone. A positive change in tension denotes contraction, a negative change indicates relaxation. The sphincter region contracted to mecholyl and to electric current but relaxed to adrenalin. The root portion of the iris contracted equally both to mecholyl and to adrenalin, but with greater force to electric current. The middle portion did not respond either to drugs or to electrical stimulation.

Examination of the microscopic anatomy of the iris indicates that radial forces developed only in the areas where there are identifiable radial muscle fibers. The radial spoke bundle of the sphincter and the radial fibers in the ciliary muscle are probably the source of the contractile forces in the pupillary and root areas. On the other hand, contractility could not be demonstrated in the so-called "dilator pupillae."

Summary

The procedures we have used are capable of measuring the amplitude of contractile forces in the iris. These methods have demonstrated radial contractile forces in the pupillary third of the iris and therefore implicate the radial spoke bundle of the sphincter pupillae. Similarly, in the root the identified radial contractility is probably due to action of the radial ciliary muscle. In the intermediate region, however, where only the dilator pupillae was present, there was no evidence of contractility at all. Therefore, it is concluded that radial contractility in the iris arises in the sphincter and ciliary muscles and not in the so-called dilator pupillae.

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Discussion

L. M. N. Bach (Tulane University, New Orleans): First of all, I want to offer my congratulations and gratitude to Dr. Apter for her splendid presentation. It is not often that we have the opportunity to hear about such elegant and succinct experiments. Personally, I have been waiting for a long time to see the evidence which she has so ably presented because I have never been convinced about the contractile properties of the so-called radial musculature of the iris.

It is indeed strange that scientists have accepted, for so many years, the rather curious statement that the "myoid" elements of the radial musculature are the only examples of muscle tissue which does not have a mesodermal origin. This statement, in itself, should have prompted someone to examine its validity.

When we examine the origin and nature of the so-called radial musculature of the iris, it becomes immediately apparent that the iris is simply special development of the photomechanical devices used by more primitive forms. Thus in frogs and fishes, it is possible to describe small myoid elements in the pigment epithelium of the retina which retract the rod or cone elements in response to various levels of illumination. Through the phylogenetic scale, we see that this mechanism is gradually replaced by an extension of the pigment epithelium beyond the ciliary body to form irides of different types. Depending on the environmental circumstances of the animal's habitat, its relationship to the sun in terms of feeding and other behavior, we find all sorts of pupil shapes appearing and the musculature arrangements of the iris developing accordingly. Least of all, do we find any significant retention of the more myoid devices simply because their function has been replaced by the iridic musculature.

There has been an old unsettled controversy concerning the participation of the sympathetically innervated iris dilators and parasympathetically innervated iris constrictors in the pupillary responses to near and far vision and to light. Current opinion indicates that inhibition of parasympathetic pupilloconstriction is the basic mechanism in pupillary dilatation in the human. In lower species, the opinion is less certain. Thus it has been shown that the pupillary reflexes persist in the absence of sympathetic innervation in the rabbit whereas it is claimed that sympathetically innervated pupillodilatation plays an important role in accommodation in the monkey and cat. This question may be better resolved by examining the irides of various species following the procedures Dr. Apter has described.

Dr. Burian (Iowa City, Iowa):

I rise to congratulate Dr. Apter on the fascinating piece of work which she has so ably presented to us.

It is of interest to note the history of the question of the existence or nonexistence of a dilator muscle in the iris. For many years the histologists have denied the existence of such a muscle but the physiologists continued to clamor that such a muscle should exist. The histologists finally have agreed to its existence and now the tables have been turned and the physiologists deny its existence. The experimental evidence presented to us today would appear to be rather strong, although the argument against a "muscular" nature of the iris muscles, because of their epithelial origin, is not valid. There are other contractile cells in the body—for example, the m. erectores pilorum—which are also of epithelial origin.

I should like to ask Dr. Apter just one question. Have you formed any hypothesis regarding the difference in the effect of adrenalin on the pupillary and ciliary part of the iris?

STIMULATION OF DYE-TEST ANTIBODIES IN HUMAN VOLUNTEERS USING HEAT-KILLED TOXOPLASMA*

JOHN R. FAIR, M.D.
Augusta, Georgia

Immunity patterns following vaccination with killed Toxoplasma have been studied in the guinea pig by Cutchins and Warren.[1] Using an antigen prepared from formaldehyde treated or sonic vibrated Toxoplasma to which a Freund-type adjuvant had been added, these authors were able to protect guinea pigs against challenge inoculation except by the intracerebral route. Both dye test and complement fixing antibodies appeared in the serum of the vaccinated animals.

Similar studies in man are suggested by the ever increasing interest in human toxoplasmosis. It is the purpose of this paper to report the appearance of dye test antibodies in humans following the injection of heat-killed whole Toxoplasma.

PROCEDURE AND RESULTS

In an early experiment, skin test antigen prepared after the method of Frenkel[2] was used in a series of 11 volunteers in an attempt to stimulate dye test antibody formation. This preparation is very dilute and, as might be expected, produced little or no response. After skin and dye tests were performed, each of the 11 subjects was given 0.5 cc. of the skin test material by subcutaneous injection at weekly intervals for three doses—a total of 1.5 cc. Two weeks after the last injection, blood was collected for the dye test. Figure 1 indicates the results. No measurable response was elicited in those individuals who gave negative skin and dye tests prior to vaccination. A slight rise in titer suggesting the recall phenomenon occurred in the case of previously infected subjects—those with positive skin and dye tests at the beginning of the experiment. In this and the succeeding studies, "before" and "after" serum specimens were tested in parallel fashion to avoid day to day technical differences in the dye test.

In a second phase of the experiment, another 11 volunteers were injected with an antigen consisting of heat-killed whole Toxoplasma in mouse peritoneal exudate diluted four times. This material was untreated except for exposure to a temperature of 56°C. for one hour on each of two successive days and the four fold dilution with physiologic saline. After proper precautions as to sterility of the vaccine were observed, each of the 11 subjects was given 0.5 cc. of the material by subcutaneous injection at weekly intervals for three doses. Two weeks after the last injection, blood was again collected for the dye test. Figure 2 indicates the results. Considerable variation will be noted. In one

NUMBER	BEFORE ANTIGEN		AFTER ANTIGEN
	skin test	dye test	dye test
1	–	–	–
2	–	–	–
3	–	–	–
4	–	–	–
5	–	–	–
6	±	–	–
7	±	+ 1:64	+ 1:128
8	+	+ 1:64	+ 1:512
9	+	+ 1:128	+ 1:512
10	+	+ 1:256	+ 1:256
11	+	+ 1:512	+ 1:1024

Fig. 1 (Fair). Results of an attempt to stimulate dye-test antibody formation in human volunteers by injection of a dilute refined antigen (skin test preparation prepared according to the method of Frenkel).

* From the Ophthailmology Division, Department of Surgery, Medical College of Georgia. This study was supported by grants from the Knights Templar Eye Foundation, the United Cerebral Palsy Research and Educational Foundation and the United States Public Health Service.

subject no response was elicited (Case 1, fig. 2). In one previously infected individual, the serum dye test titer remained unchanged at 1:1024. In the nine remaining subjects dye test antibodies appeared or, as in Cases 9 and 10, increased from previous levels by several tubes in the test line.

Because of the natural objections to a "raw" antigen such as that described in the previous experiment, a refined but concentrated preparation was obtained* for use in a third group of volunteers. This material was made according to Frenkel's description of the preparation of skin test antigen but was diluted only 50 times rather than the usual 1,000 times, being, then, 20 times more concentrated than the skin test antigen used as a vaccine in the initial phase of the study. Three 0.5 cc. doses of this material given at weekly intervals stimulated no dye test antibody formation except in one previously infected subject, as can be seen in Figure 3. Again, blood for follow-up dye test was drawn two weeks after the third dose of vaccine. Because their methods of preparation differed widely and were carried out in different laboratories, this last antigen and the mouse peritoneal exudate diluted four times used in the previous experiment cannot be compared on the basis of number of organisms represented per unit of volume. No parasite counts were made on exudate except that only material containing a rich yield of organisms was used.

NUMBER	BEFORE ANTIGEN		AFTER ANTIGEN
	skin test	dye test	dye test
1	–	–	–
2	–	–	+ 1:2048
3	–	–	+ 1:16,384
4	–	–	+ 1:32
5	–	–	+ 1:128
6	–	–	+ 1:512
7	–	–	+ 1:1024
8	–	–	+ 1:64
9	–	+ 1:4	+ 1:128
10	+	+ 1:128	+ 1:1024
11	±	+ 1:1024	+ 1:1024

Fig. 2 (Fair). Stimulation of dye-test antibody formation in human volunteers by injection of heat-killed Toxoplasma in mouse peritoneal exudate diluted four times.

CASE NO.	DYE TEST	
	BEFORE VACCINATION	AFTER VACCINATION
1	Neg.	Neg.
2	Neg.	Neg.
3	Neg.	Neg.
4	Neg.	Neg.
5	Neg.	Neg.
6	Neg.	Neg.
7	Neg.	Neg.
8	Neg.	Neg.
9	Neg.	Neg.
10	+ 1:256	+ 1:2048

Fig. 3 (Fair). Results of an attempt to stimulate dye-test antibody formation in human volunteers, using a concentrated refined antigen.

Discussion

The immunology of toxoplasmosis is connected directly with the prevention and treatment of chorioretinitis. Clinical evidence linking toxoplasmosis and chorioretinitis continues to accumulate. The etiologic relationship between the congenital form of chorioretinitis and toxoplasmosis is particularly clear. Figure 4 illustrates the results of a serologic investigation of 71 cases of congenital chorioretinitis in which the mothers were available for study. Dye tests were positive in both patient and mother in 80% of the 71 cases. Because of the prevalence of past or persistent infection in the general population, a combination of positive tests in mother and offspring has much greater diagnostic significance than a positive test in patient alone.

* Both dilute and concentrated antigen of the refined variety were obtained from the Eli Lilly Co., Indianapolis, Indiana.

TOTAL	DYE TEST			
	PATIENT POS. MOTHER POS.	PATIENT NEG. MOTHER NEG.	PATIENT POS. MOTHER NEG.	PATIENT NEG. MOTHER POS.
71	57 (80%)	8 (11%)	6 (8%)	0

Fig. 4 (Fair). Results of dye test for toxoplasmosis in patients and mothers in 71 cases of congenital chorioretinitis.

Chorioretinitis is the most constant finding in congenital toxoplasmosis. Further, in the majority of cases of congenital chorioretinitis due to toxoplasmosis, the eye inflammation is unaccompanied by other signs of the primary disease. That is, it seems likely that the commonest form of congenital toxoplasmosis is that in which only the eyes are seriously involved. If this is true, then congenital toxoplasmosis is much more common than previously thought and many cases of bilateral or recurrent chorioretinitis in older children and adults may be only the ocular manifestations of congenital infection.[3-6] The prevalence of congenital toxoplasmosis in which chorioretinitis is the only clinical manifestation helps to explain the discrepency between the observed and estimated frequencies of the congenital disease.

The obvious treatment of congenital toxoplasmosis is its prevention. Until we learn the method of transmission of the acquired infection, we are left with the possibility of immunizing previously uninfected (non-immune) pregnant women. Congenital toxoplasmosis due to chronic or latent infection in mothers apparently is not a problem in humans. What is required is the production of an active immunity which will prevent acquisition of the infection during pregnancy.

Admittedly, there may be no connection between immunity and either dye test or complement fixing antibodies. The laboratory mouse develops dye test antibodies following vaccination with whole killed Toxoplasma but succumbs to challenge just as readily as do unvaccinated controls. In the study by Cutchins and Warren mentioned previously, guinea pigs developed dye test antibodies but no immunity when vaccinated with whole or fragmented parasites alone. Both complement fixing and "protective" antibodies (if these are different) appeared when adjuvant was combined with the vaccine. A disturbing fact was the dissemination and persistence of viable parasites in the various tissues of the host in the presences of high levels of complement fixing antibodies in both vaccinated and convalescent animals.

Studies in humans should be extended to include measurement of complement fixing antibodies following vaccination since these seem to appear simultaneously with immunity in guinea pigs. Other topics for study which immediately suggest themselves are:

1. The use of larger doses of vaccine with measurements of both dye test and complement fixing antibody response.
2. Trial of vaccine-adjuvant combinations if larger doses of vaccine alone do not result in higher antibody levels.
3. Accurate comparison of the antigenic strength of vaccines made of whole and fragmented organisms based upon parasite counts on pooled exudate.
4. The use of more natural routes of infection after vaccination of the laboratory mouse.
5. Prevention of congenital toxoplasmosis in laboratory animals by vaccination.

As the frequency of congenital toxoplasmosis is better appreciated, the importance of its prevention will become apparent. The

possibility of immunizing previously uninfected pregnant women seems to be a natural approach to the problem. In this matter, the ophthalmologist should lead the way.

Summary

1. Vaccination of human volunteers with whole heat-killed Toxoplasma resulted in the production of significant levels of dye test antibodies. The possibility of preventing congenital toxoplasmosis by immunizing previously uninfected (nonimmune) pregnant women is discussed. Related subjects for future study are listed.

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OBSERVATIONS ON THE ETIOLOGY OF TRACHOMA*

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Introduction

The Aramco Trachoma Research Program was initiated in October, 1954, and has been reported upon very briefly at previous meetings.[1-3] The program is concerned with trachoma and certain other diseases of the eye and its principal objectives are the development of more accurate diagnostic methods, the clarification of etiology, and the attempt to establish means for prevention. The program has two laboratories in operation, one in Dhahran, Saudi Arabia, and the other in Boston.

Several thousand specimens for microscopic examination have been taken from the conjunctivas of patients in Saudi Arabia and several other countries, particularly Yugoslavia and Portugal. In addition to the morphologic studies approximately 2,000 specimens have been obtained and preserved at −60°C. Thus far, 948 conjunctival scrapings have been tested by inoculation of culture media for isolation of bacteria, and by inoculation of human epithelial cells for isolation of viruses. The remainder are being screened as rapidly as laboratory facilities permit. In a few instances, serum specimens have been obtained but this procedure is difficult in Saudi Arabia except on hospitalized patients. Studies have concentrated on the natural history of diseases of the eye, in the absence of specific therapy.

From the conjunctival scrapings of Saudi Arab patients having clinical findings con-

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sistent with trachoma, the staff members of the program have isolated various agents, including 65 strains of adenoviruses, numerous strains of bacteria belonging to four main species, and 14 strains of elementary bodies. The relationship of the various agents thus shown to be present in diseased eyes in Saudi Arabia to the actual pathologic processes requires further clarification.

It is the purpose of this paper to review the general findings in the program and to note some of the properties of the agents which have been obtained in this investigation of trachoma and related diseases of the eye.

Bacteria

Conjunctivitides are almost ubiquitous in Saudi Arabia. It is uncommon to find a sterile bacterial culture of the conjunctival surfaces taken from infants older than two weeks, or from older children and adults. Four species of bacteria have been cultured: One species, a gram-negative diplococcus, produces a clinically distinct syndrome which occurs sporadically in sharp outbreaks (this clinical syndrome may be closely related to a conjunctivitis occurring seasonally in Egypt and reported to be caused by the gonococcus). The other three common species of bacteria are found in normal eyes as well as trachomatous eyes in Saudi Arabia. One of these three species is a diphtheroid, Corynebacterium xerosis. Another is an atypical streptococcus. The third micro-organism belongs in the hemophilus group and is related to H. influenzae and H. aegyptius (Koch-Weeks). These organisms may induce acute conjunctivitis, but may also be present in eyes which appear normal macroscopically. It remains to be determined what role they play in the scarring and deformities consequent to severe trachoma in Saudi Arabia.

Adenoviruses

Since the inception of the program in 1954, eye scrapings from 948 Saudi Arab patients diagnosed as having clinical trachoma have been tested in tissue culture. From these isolation attempts, 67 strains of viruses were isolated; two proved to be Coxsackie strains B1 and B2 while all the remainder fell in to the group known as adenoviruses.

Methods used to obtain specimens, types of preserving fluids, interval and manner of storage, and technique of tissue culture isolation are reported elsewhere.[4,5] The identification of adenovirus types was made primarily by neutralization tests with specific rabbit antisera[6] and in many instances also by the use of a recently reported hemagglutination and hemagglutination-inhibition test.[7] All 65 virus isolates fixed complement in the presence of known positive adenovirus antiserum.

The frequency of isolation of adenoviruses in Saudi Arabia is closely related to the age of the patient and the season in which the isolation attempt is made. During the summer months of April through September, 60 of the 65, or 92 percent, of the viral strains were isolated. These 60 isolations were made from a total of 451 isolation attempts or a rate of 13.3 percent. In contrast, during the winter months of October through March, 495 isolation attempts were made with only five successful viral isolations or a rate of one percent.

Of the 62 isolations where the age was known, 81 percent were from children under the age of two years; 90 percent were under the age of three years. Only six isolations of virus were made from children three years of age or over; these six isolations resulted from a total of 467 isolation attempts giving an isolation rate of 1.3 percent for children three years of age and older. In contrast, from 448 isolation attempts made from children who were less than three years of age, 56 successful isolations resulted or an isolation rate of 12.5 percent. It is of interest that four of the six isolations in children three years of age or over were of type 3. In fact, type 3 is the only adenovirus recovered from children over the age of three years in

our tests, with the single exception of one type 15. Most Saudi Arab children obviously are exposed to several adenoviruses before the age of three years.

Fourteen different types were obtained (including one untyped). Standard types 3, 8, 15, 17 and new type Bar-2[7] preponderate. It is of interest that only two strains of type 7 and three strains of type 4 were obtained. From eight patients scrapings were made of each eye and treated as separate entities. In all eight cases, the same virus was obtained from each pair of eyes. Types 1, 2, 5, 10-14 and 18 were not encountered.

Experiments in Hongkong[5] indicated that two of the Saudi Arab adenovirus strains were able to produce follicular conjunctivitis in man, and that the ensuing syndrome was not distinguishable on clinical grounds from naturally occurring early trachoma in the same community.

Further studies are needed to clarify the role of the different adenovirus types in the symptom complex of trachoma in Saudi Arabia.

Elementary bodies

In May, 1958, a paper by Tang, et al.[8] in English became available which reported the isolation of strains of elementary bodies by the inoculation of chick embryos, using a modification of the technic of Cox[9] and Macchiavello.[10] A British scientist visiting the communist Chinese research laboratories in 1957 obtained the Peiping virus and brought it to Dr. Collier of the Lister Institutes, London, late in 1957. Dr. Collier soon confirmed the Chinese isolations.[11-13] In the Boston laboratory, Tang's modification of the egg technique has been applied to eye scrapings from Saudi Arab patients; 14 different strains of elementary bodies have been isolated. The scrapings had been stored at −60°C. for periods up to 20 months.

The technique of isolating strains consists of the injection of the yolk sac of the embryonated hen's egg with material obtained from the conjunctival sac of the eyes of trachoma patients. Thus far material has been selected for trial which has shown the presence of numerous inclusions in the conjunctival cells. The material is suspended in a solution of streptomycin and held for at least one hour at 4°C. before injection into the yolk sac of six- or seven-day-old chick embryos. (The use of approximately 1,000 μ of streptomycin per egg is the modification introduced by the Chinese workers.)

When there are large numbers of elementary bodies in the material obtained from the conjunctival scrapings, it is possible that the chick embryos inoculated therewith may die on the eighth to ninth day after inoculation, and that elementary bodies may then be abundant in the yolk sac membranes. If the material from the patient's eye contains relatively small quantities of elementary bodies, it may require up to four or five blind passages in eggs before elementary bodies can be observed in the microscopic preparations.

Up to the present time five of the 14 strains of elementary bodies have been carried through at least five passages in chick embryos and one strain has gone 19 serial passages; no significant difference in character or profuseness of growth has been noted between the strains. The data from the first strain to be isolated are given in detail below.

Characterization of the Saudi Arab Strain 1. In July, 1957, in the village of Al Omran, in the Hofuf oasis, an 18-month-old girl was examined and found to have a clinical diagnosis of trachoma II. A scraping of her conjunctiva was taken and part was used for microscopic examination; the Giemsa-stained smear showed numerous inclusions in the conjunctiva cells. The remainder of the scraping was transferred to a small screw cap vial, frozen at −60°C. in an electric cabinet for the ensuing 10 months. In May, 1958, the vial was thawed and the material processed as already described. By the third passage in eggs, the chick embryos died on the fifth or sixth postinoculation day. Large numbers of elementary bodies were

visible in yolk sac smears. This strain was designated as SA-1, the Hofuf strain. The viral particles of SA-1 stain red with Macchiavello's stain and pinkish violet with Giemsa. Their appearance under the microscope is similar to the appearance of the viral particles of psittacosis and other members of the psittacosis-ornithosis group.

Cultures of SA-1 suspensions for bacteria and pleuropneumonia-like organisms showed no microorganisms.

Test of SA-1 in a Chimpanzee. A concentrated suspension of SA-1 was inoculated into the conjunctival sac of a chimpanzee in June 1958. Forty-eight hours later, acute inflammation of the conjunctivae was apparent. Approximately 72 hours postinoculation, microscopic examinations of the conjunctival cells in the scrapings revealed the inclusions which are considered diagnostic of the form of trachoma which is induced by elementary bodies. The chimpanzee recovered spontaneously and her eye was grossly normal in appearance at the end of a week.

Preliminary Serologic Tests with SA-1. An antigen was prepared from SA-1 yolk sac material by purifying and concentrating the viral particles. This antigen showed high complement fixing titers with known positive antipsittacosis serum as well as with several sera from patients who had been diagnosed both clinically and microscopically as having classical trachoma. This observation is adequate to make a preliminary classification of the SA-1 Hofuf strain as a virus of the psittacosis-ornithosis group. It is of great importance that the strain is *not* contaminated with pleuropneumonia-like organisms which had interrupted our egg experiments three years previously.

Success in propagating elementary bodies in chick embryos represents a great step forward in trachoma research, but it is not possible to immunize chick embryos nor to use them for assaying different preparations which are being considered as vaccines. Consequently, an intensive search was begun to find a small laboratory animal which could be used as a tool to study the problems associated with immunizing against the elementary body virus. Three species of rodent, the white mouse, the Mongolian gerbille, and the Egyptian spiny mouse were found to succumb within one to 24 hours following intravenous inoculations of concentrated suspensions of elementary bodies of the Hofuf or SA-1 strain.[3] This rapid lethal effect in the mouse is attributed to a toxin associated with the elementary bodies. It is eliminated by heating the virus at 56°C. for 30 minutes and is similar in many respects to the toxic properties of certain other intracellular parasites such as typhus rickettsiae and members of the psittacosis-lymphogranuloma-ornithosis group of viruses.

The role of elementary bodies and inclusions in trachoma has been much debated. There are wide differences in the occurrence of inclusions in conjunctival scrapings of clinically identical disease states between different geographical areas, or indeed, between two villages only a kilometer apart. It is hoped that some light on this question may be shed by using the toxic phenomenon for comparing the immunologic characteristics of elementary bodies obtained from different geographical areas. It is essential to establish whether there is one single variety, or many different types of elementary bodies, before it is possible to select strains for vaccines or to evaluate the role of a vaccine in prevention of trachoma in man.

Summary

In 1954, the Medical Department of the Arabian American Oil Company and the Department of Microbiology of the Harvard School of Public Health began a co-operative study on trachoma and certain other diseases of the eye in Saudi Arabia. The principal objectives of the program have been the development of more accurate diagnostic methods, the clarification of etiology and the attempts to find means for prevention. The program has two laboratories in operation,

one at the Aramco Health Center in Dhahran, Saudi Arabia, and the other at the Harvard School of Public Health, Boston, Massachusetts.

Several thousand specimens for microscopic examination have been taken from the conjunctivas of patients in Saudi Arabia and several other countries, particularly Yugoslavia and Portugal. Thus far 948 conjunctival scrapings have been tested by bacterial cultures, by inoculation of human epithelial cells for isolation of viruses, or by the inoculation of chick embryos for the detection of elementary bodies.

The results of the program during the first four years of its operation are presented, including a summary of the various strains of adenoviruses, bacteria and elementary bodies which have been isolated from conjunctival scrapings of Saudi Arab patients having clinical findings consistent with trachoma. The paper emphasizes the need for further studies to determine the actual role of the adenoviruses, bacteria and elementary bodies, acting alone or in concert one with the others, in producing the severe scarring disease which is typical of trachoma in certain geographic areas.

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THE USE OF CORTISONE IN ESTABLISHING EXPERIMENTAL FUNGAL KERATITIS IN RATS*

A PRELIMINARY REPORT

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INTRODUCTION

Serious problems have arisen from the indiscriminate use of cortisone preparations in the treatment of ocular infections, as can be shown by the enhancement of fungal keratitis. Several reported clinical observations have borne this out,[1–4] in particular the studies of Ley[5] have given experimental impetus to these observations. The inherent dangers of steroid treatment in ocular infections have been emphasized by Allen,[6] Offret and Massin,[7] and Haggerty, et al.[8]

The purpose of this study, therefore, is to devise an adequate and consistently reproducible experimental system for the establishment of fungal corneal ulcers whereby cortisone preparations serve as adjuncts or accessory factors if necessary. Ideally such a system involves the initiation of fungal keratitis without the use of cortisone, however, at the moment this apparently is not feasible. Such a system might prove to be of value in the study of the pathology, experimental therapy and mechanisms by which cortisone potentiates fungal keratitis.

MATERIALS AND METHODS

The fungi serving as test agents in these experiments are as follows: Cephalosporium sp., Aspergillus fungatus, Aspergillus flavus, Aspergillus cereus and two Fusarium species. Cephalosporium was isolated from a clinical case of keratitis[9] while the remaining fungi were obtained from the fungal collection of Dr. L. Friedman. Cephalosporium was the agent of choice, in the majority of the experiments. This fungus was grown on casamino yeast or Saboraud's medium for six or seven days at room temperature. Several plates or slant cultures of the fungus were gently washed with 3.0 to 5.0 ml. aliquotes of sterile saline, and pooled in a sterile screw cap bottle and refrigerated. This preparation contained a moderately heavy spore suspension with relatively few mycelial filaments. In early experiments, the spore suspensions were standardized by a dilution-plating method to contain 40,000 to 85,000 viable particles per ml. In later experiments the spore suspensions were standardized by visual turbidimetric procedures. The suspensions were periodically checked for contamination. Mycelial inocula were prepared by vigorously shaking a small portion of the young cottony aerial growth with glass beads.

Prednisolone Sodium Hemisuccinate (Meticortelone®, Schering) and 1.5 percent ophthalmic suspension of hydrocortisone acetate (Cortef acetate®, Upjohn) were the steroid preparations employed in this study.

In the group A experiments, mature rabbits, average weight, 3.5 kg. were used while mature rats, average weight, 300 gm. served as the experimental animals in group B.

The animals in the group A experiments were anesthetized with 0.5 to 0.8 ml. of intravenously administered nembutal while the rats in the group B experiments were placed under ether anesthesia. The inoculum consisted of 0.05 to 0.1 ml. of the saline spore suspension which was injected intracorneally employing a tuberculin syringe equipped with a 25- or 30-gauge needle.

The cortisone preparations were given intravenously, subcutaneously, or applied topically. The routes of administration of the

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compounds and the duration of cortisone treatment are listed below in the results of the respective experiments. The Gomori and Gram stains were made on preparations for histological examination. Scrapings from ulcers were cultured on appropriate media for fungal and bacterial controls.

Results

Group A

I. One mature rabbit was pretreated with intravenous prednisolone for 10 days with a dosage schedule of 2.5 mg. for the first five days. The dosage was doubled for the remaining five days culminating in a 25 mg. aliquot. The 10th day bilateral intracorneal injection of the Cephalosporium spore suspension was made. Ten mg. of prednisolone were given at approximately weekly intervals following inoculation.

At the end of approximately 21 days the right eye showed a small area of scarring (1.0 mm. diameter) and opacity at the site of inoculation while the left eye remained clear. Both corneas were inoculated in the control rabbit and prednisolone was not given; there appeared only a slight opacity in the right eye which cleared in a few days. Histologic examination did not show evidence of fungal keratitis in the control or cortisone treated animals.

II. The second experiment involved bilateral intracorneal inoculation of the Cephalosporium spore suspension and the application of prednisolone topically five drops twice daily for 14 days to the right eye. The untreated left eye served as the control. The treated eye developed an ulcer which was shown to be bacterial in origin when cultured on blood agar medium. The control eye remained clear. No fungal keratitis was initiated in this experiment.

III. One animal was inoculated intracorneally in each eye with a young mycelium suspension and another animal was similarly inoculated and treated with prednisolone which was applied topically twice daily for 20 days. No evidence of corneal infection could be detected in either the control or the treated animal.

IV. The corneas of a mature rabbit were abraded slightly with a 26-gauge needle and the spore suspension rubbed into the traumatized eyes; the right eye remained untreated with topical cortisone. There was no evidence of infection at the end of this period in the treated or the control eye.

V. An animal was inoculated with spores bilaterally near the superior border of the limbus and observed for 14 days. No cortisone was applied, and no infection resulted.

In each of the above experiments no fungal corneal ulcer could be established with or without the use of prednisolone. These preliminary studies tend to corroborate Ley's[5] unsuccessful attempts at establishing Cephalosporium keratitis in mature rabbits. The subconjunctival injection of cortisone was not attempted in this instance. It is interesting to note that the growth of Cephalosporium was shown to be greatly retarded and scant on media supplemented with rabbit serum. There is possibly a humoral factor in the rabbit serum which inhibits growth consequently preventing infections of this species in rabbits.

The unsuccessful attempts at establishing fungal ulcers in the rabbit necessitated the use of another type of experimental animal. Rats were chosen for group B experiments. The problem of inoculating such small eyes was solved by means of 30 gauge needles.

Group B

I. Both corneas of three mature rats were injected with the Cephalosporium spore suspension and the inoculated eyes were observed for two weeks. At the end of this period there was no evidence of corneal opacity or ulceration. Only a slight opacity could be noted immediately following inoculation; this cleared within three or four days. Histopathological stains of these preparations were negative.

II. The second experiment involved inoculation of a young mycelial suspension into

the right eyes of two animals while a heat-killed aliquot of the same preparation was introduced into the left eyes. Maximal opacity involving nearly the entire cornea and ulceration of the right eye of one animal was observed in 48 hours. Questionable ulceration was present in the right eye of the other animal. The left eyes merely showed some degree of corneal opacity and no ulceration at the end of one week.

III. Four animals were pretreated with prednisolone administered subcutaneously (dosage schedule of 25 mg. per day for two consecutive days, totaling 50 mg.). The third day bilateral intracorneal injection of Cephalosporium spore suspensions were made. In seven of the eight eyes, extensive corneal opacification, ulceration, exudate formation, perforation, lens protrusion and slight hemorrhage into the anterior chamber appeared in 48 to 72 hours. The area of ulceration and perforation was 1.0 to 2.0 mm. in diameter while opacification covered the entire cornea. The pathologic process was observed within 24 hours after inoculation. Scrapings of three of the infected corneas were cultured on casamino yeast extract, all of which were positive for Cephalosporium. Histologic examination of the sections revealed mycelia growing into the corneal stroma.

In this experiment the control animal was similarly pretreated, except a heat killed spore suspension served as the inoculum. Only a small area of opacity appeared initially and this cleared within three or four days.

IV. An experiment was conducted to determine the minimal amount of prednisolone necessary to induce and maintain the infection. It was found that the total dosage of prednisolone could be reduced to 25 mg. (12.5 mg. per day over a two-day period) and the corneal lesions still be observed, however, these lesions were not as progressive and extensive as those observed resulting from a total dose of 50 mg. Smaller drug dosages did not initiate or maintain the fungal infection.

V. The effect of topically applied cortisone was next studied. The right corneas of two rats were injected with a viable spore suspension while the left corneas received a heat killed spore suspension. All eyes were treated with one or two drops of 1.5-percent ophthalmic suspension of hydrocortisone acetate twice daily. Marked ulceration and perforation resulted within a 24 to 48 hour interval as seen in Experiment B, III in the treated eyes while the eyes receiving the heat-killed spore suspension showed only minimal opacity and ultimate clearing. Histopathologic examination of sections of these ulcers were positive for the fungus.

VI. Six animals were inoculated with viable Cephalosporium spores and treated as in group B part V. However, in order to obtain maximum involvement of the eye within four or five days it was necessary to maintain the infection by early application of the cortisone. If the cortisone treatment were stopped, the area of ulceration did not progress and rapid organization scarring and vascularization ensued. The results of these experiments are summarized in Table 1.

The preceding preliminary studies have been applied to several other fungi species, however, these studies as yet, are not as inclusive as those concerned with the Cephalosporium sp. Several species of fungi usually considered nonpathogenic under natural conditions were selected for these studies. The fungi being investigated are Fusarium sp., Fusarium roseum, Aspergillus fumigatus, A. cereus and A. flavus. A Curvilarium sp. isolated from a clinical case of corneal ulcer recently seen in this department has also been included in the above list of fungal agents. The materials and methods for this group of experiments are essentially the same as those for group B differing only slightly in the preparation of the spore suspensions. When the suspensions were prepared, several glass beads were allowed to roll gently across the surface of the slant in order to obtain some degree of emulsification of the spores.

Two mature rats were tested with each

TABLE 1

SUMMARY OF RESULTS FROM THE GROUP B EXPERIMENTS

Experiment No.	No. Animals	Eye	Inoculum	Cortisone Preparation	Route of Treatment	No. of Ulcers	Cultures		Histologic Examination	
							Total	+	Total	+
I	3	O.D.	Viable spores	—	—	0	0	0	1	0
		O.S.	Viable spores	—	—	0	0	0	1	0
II	2	O.D.	Viable mycelium	—	—	1	1	0	2	0
		O.S.	Heat-killed mycelium	—	—	0	0	0	2	0
III	4	O.D.	Viable spores	Prednisolone	Subcutaneous	3	1	1	1	1
		O.S.	Viable spores	Prednisolone	Subcutaneous	4	2	2	2	2
	1	O.D.	Heat-killed spores	Prednisolone	Subcutaneous	0	0	0	0	0
		O.S.	Heat-killed spores	Prednisolone	Subcutaneous	0	0	0	0	0
V	2	O.D.	Viable Spores	Hydrocortisone Acetate	Topical	2	0	0	2	2
		O.S.	Heat-killed spores	Hydrocortisone Acetate	Topical	0	0	0	2	0
VI	6	O.D.	Viable spores	Hydrocortisone Acetate	Topical	5	0	0	0	0
		O.S.	Viable Spores	Hydrocortisone Acetate	Topical	6	0	0	0	0

fungus species. Intracorneal injection of viable spore suspensions were made in the right eyes while corneas of the left eyes were injected with a heat-killed spore suspension of the respective fungus species. One animal of each set received ophthalmic drops of hydrocortisone twice daily. The other animal of each set remained untreated thus serving as the control.

Within 24 to 48 hours opacification and extensive ulceration developed in the right eyes of the treated animals which had been inoculated with the three Aspergillus sp. The right eyes of the tw Fusarium sp.-treated animals developed only a slight degree of opacification and a small ulcer both of which cleared in approximately seven days. No ulcers developed in the control eyes. Histopathologic examination of the Aspergillus fumigatus ulcer was positive for fungi.

The uniformly high incidence of ulcers in treated rat eyes as demonstrated in this group of experiments offers some support to the idea that it may be possible to establish many types of fungal ulcers in the rat. This animal may therefore serve as the "universal" experimental host in studies concerning experimental fungal ulcers. It is interesting to note that Ley has been successful in establishing fungal corneal ulcers in rabbits with Aspergillus terreus, A. fumigatus, Allescheria boydii and Candida albicans by means of subconjunctival cortisone injections. In our studies no attempt was made to establish these infections in rabbits.

Inquiry into the mechanism of the observed corneal destruction in Cephalosporium keratitis was made. Since an enzymatic protein has been shown to be the active factor in corneal destruction in Pseudomonas ulcers,[10] it was postulated that such an en-

zyme might be the active agent in fungal ulcers.

This problem has been approached with a few experiments directed towards determining the presence or absence of an enzyme which will cause corneal damage resulting in ulcerations. The liquid medium in which bulk quantities of Cephalosporium were grown for two weeks was injected into the corneas of rabbits. No ulceration or corneal damage was observed other than some opacification. The mycelial extract was prepared by grinding the mycelin with powdered glass and extracting the material with saline. The crude saline mycelial extract was inoculated intracorneally into the eyes of rabbits. Opacification, exudative liquefaction and ulceration were noted within four hours. Fractionation of this crude extract by precipitation with various concentrations of ammonium sulfate solution yielded a fraction which possessed ulcerative activity. These preliminary enzymatic studies indicate there is a protein factor which is responsible for the corneal destruction observed in Cephalosporium keratitis. Attempts are in progress to determine the manner by which this factor is able to destroy normal corneal tissue. Preliminary experiments indicate that this fungal factor is not the same as the corneal damaging factor of Pseudomonas.

Discussion

Though the number of animals in each of the experiments admittedly was small, the results obtained for these experimental groups were consistent and therefore considered reliable.

The failure to establish Cephalosporium corneal ulcers in rabbits with or without the use of prednisolone applied topically or administered systemically over a period of two or three weeks, is significant in that it confirms the unsuccessful attempts of such investigators as Ley and Bedell[11] in this endeavor. The latter investigator did not use cortisone.

As stated earlier there is the possibility that a humoral agent exists in the serum of this animal species which contributes to the extreme degree of resistance to Cephalosporium infections. If such a factor is present it is apparently not altered by the administration of cortisones. On the other hand, the resistance may not lie in serum but in the corneal tissue itself. However, the evidence obtained from the preliminary fractionation studies of mycelial extracts indicates that rabbit cornea is capable of destruction by some type of enzymatic protein elaborated by the fungus. The problem apparently resides in the initial spore germination and proliferative phase and remains to be investigated.

Experiments listed for group B where rats served as the experimental animal proved to be the most rewarding.

Ulcers could not be established by intracorneal spore inoculation in a total of six untreated eyes. This comprised the initial control group. However, eyes under the influence of cortisone (animals systemically treated with prednisolone or hydrocortisone acetate applied topically) and inoculated with viable spores, showed an extremely high percentage of ulceration. A total of 22 eyes were subjected to cortisone treatment out of which 20 developed ulcerations giving an approximate 91-percent ulcer incidence. The inoculum for the control eyes in the cortisone treated group of animals consisted of heat-killed spores. In the total of four such eyes no ulcers appeared. Even though the control group was small, when one considers the total group of control eyes from experiments I, III and V which includes eyes untreated and inoculated with viable spores and those treated with cortisone and inoculated with heated spores, the number of controls appears to be reliable. Thus, in a total of 10 control eyes no fungal ulcers developed. A summary of these results is given in Table 2. The few scraping cultures which were taken were shown to be positive and these findings were correlated with the presence of fungal proliferation as

TABLE 2

ULCER INCIDENCE IN THE CONTROL AND EXPERIMENTAL EYES

Control					Experimental		
Untreated	Killed Spores Treated	Total	No. Ulcers	% Ulcers	Total	No. Ulcers	% Ulcers
6	4	10	0	0	22	20	91

demonstrated histopathologically (table 1).

The resistance to corneal infection in the rat is apparently lowered by cortisone, which may be due to a decreased inflammatory response resulting in germination and growth of the spores.

Solution of the problem regarding the mechanism of corneal damage as seen in Cephalosporium keratitis may be found by identification and isolation of the enzymatic factor responsible for the tissue damage.

SUMMARY AND CONCLUSION

1. Cephalosporium keratitis could not be experimentally induced in untreated or cortisone treated mature rabbits by intracorneal inoculation of a saline spore suspension.

2. Cephalosporium keratitis could be induced in mature rats treated either systemically or topically with cortisone preparations after intracorneal injection of spores. An ulcer incidence of 91 percent was obtained in the treated eyes while no ulcer incidence was observed in the control eyes.

3. Aspergillus and Fusarium keratitis was established in cortisone treated rats.

4. Preliminary fractionation studies of Cephalosporium mycelial extracts suggest there may be an enzyme responsible for corneal destruction.

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CHANGES IN CORNEAL ASTIGMATISM OBSERVED FOLLOWING SURFACE DIATHERMY TO RABBIT CORNEAS*

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From time to time investigators have attempted to alter corneal astigmatism by various operative procedures.[1-5] This paper reports a preliminary attempt to alter the corneal curve of rabbit eyes by application of limbal diathermy.

METHOD

Rabbits, preferably not albinos, were selected and refracted by streak retinoscopy under atropine. After sufficient practice it was felt that this could be done to within one half of a diopter and required no anesthesia or restraint placed upon the animal. Observations with an ophthalmometer were made but not used due to the irregular and constantly changing character of the mire.

Cats were discarded because of technical difficulties. Retinoscopy was confused by the multiplicity of the reflexes and the animals would not hold still enough to permit observation with the ophthalmometer, although a good mire could be observed momentarily. With anesthesia the eyes closed and could not be easily opened.

After determining the refraction the animal was anesthetized and diathermy applied either in the 90- or 180-degree meridian using a ball electrode. A Walker-Rose diathermy machine was employed, being set between 40 and 60 in each case. Ten animals were selected. Two died following diathermy and on two others partially penetrating diathermy was tried. These are not reported since in each case at least one application perforated the cornea complicating the eye.

Refractions were performed immediately after diathermy, the following day, and weekly thereafter for several weeks. After the first few weeks this period was extended to monthly or less frequently, and at 14 months the experiment was terminated.

No regard was given to possible damage done to intraocular structures, but in some cases what appeared to be peripheral iris atrophy was noted under the diathermy scars. Later, representative eyes will be sectioned for study.

RESULTS

The results are summarized in Figure 1. They show that, in most cases, an increase in the corneal curvature in the meridian of the diathermy occurred within a few days. Few, however, maintained this level. One eye continued to show an increase of astigmatism on each successive refraction, and both eyes on rabbit 6 developed a maximum effect opposite to that expected which then rapidly returned to prediathermy levels. Also noted was an immediate relative myopia presumed to be due to direct stimulation of the ciliary muscle. This effect was transient and not observed on the day following diathermy.

On the graph, the numerator of the fraction shown below the column for each eye is the product of the machine setting and duration of the application in seconds. The denominator is the machine setting. On the extreme left the figure opposite the fraction shows the area of application, such as the 12-, 6-, 3-, or 9-o'clock position.

From this one can get some idea of the total amount of current used as well as how it was applied.

CONCLUSIONS

It is concluded that in rabbit eyes a corneal astigmatism can be produced or modified by limbal diathermy. In eight out of 12 eyes definite maximum astigmatic effect was noted within the first two weeks. Subse-

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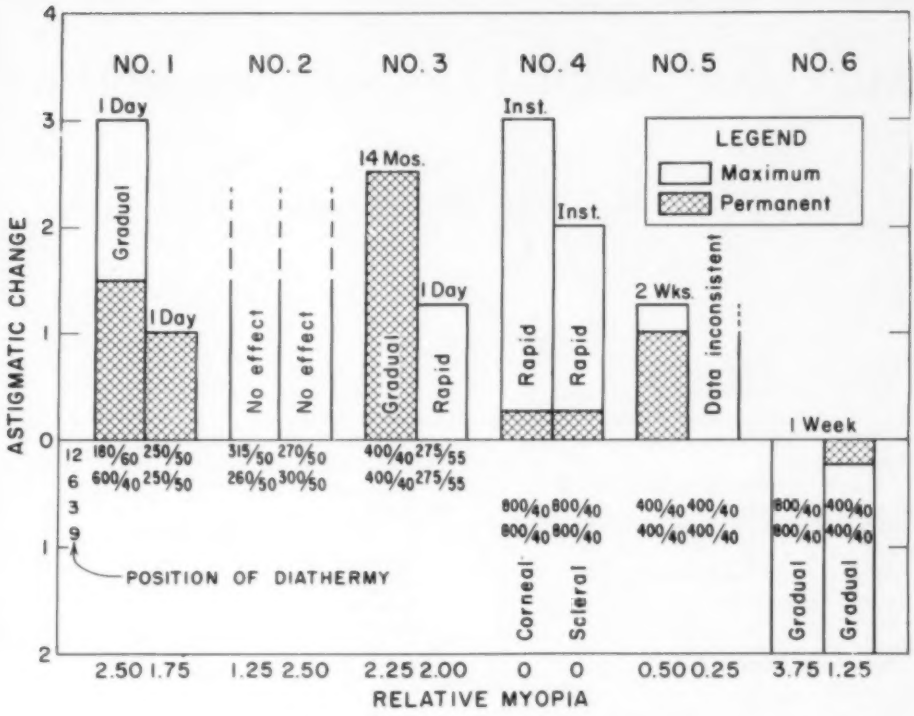

Fig. 1 (Winter). Summary of results.

quently, six of these became significantly less, one returning to a level of approximately one half of its maximum and four returning to a level very near that observed prior to diathermy. Two eyes remained essentially unchanged from their maximum effect. In one eye astigmatism increased continuously during the experiment.

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